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EVALUATION OF BIOLOGICAL AND MALE REPRODUCTIVE FUNCTION RESPONSES TO POTENTIAL LEAD EXPOSURES IN 155 MM HOWITZER CREWMEN

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A collaborative pilot study between the U.S. Army Biomedical Research and Development Laboratory and the National Institute for Occupational Safety and Health was designed to assess fecundity of male artillery soldiers with potential exposures to airborne lead aerosols. Many soldiers in the initial control population reported possible job-related microwave exposure as radar equipment operators. As a result, a third group of soldiers without potential for lead or microwave exposure, but with similar duty-associated environmental exposure conditions, was selected as a comparison population. Blood hormone levels and semen analyses were conducted on artillerymen (n=30), radar equipment operators (n=20), and the comparison group (n=31). Analysis of the questionnaire information revealed that concern about fertility problems motivated participation of some soldiers with potential artillery or microwave exposures. Data analysis was complicated by the small study population size and the confounding variable of perceived infertility. Although

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the small number of subjects and infertility concerns somewhat compromise the statistical power and general applicability of the study, several statistically significant findings were identified. Artillerymen who perceived a possible fertility problem demonstrated lower sperm counts/ejaculate of borderline significance (p=0.067) and significantly lower sperm/ml (p=0.014) than the comparison group. Individuals with potential microwave exposures demonstrated lower sperm counts/ml (p=0.0085) and sperm counts/ejaculate (p=0.027) than the comparison group. Individuals from the artillery and radar groups who perceived a fertility problem had fewer morphologically normal sperm (artillery p=0.019 and radar p=0.032) than the comparison group. The mean seminal fluid fructose level was significantly higher in the artillerymen (p=0.027) than for the comparison group. Most other variables used to assess endocrine and sperm cell function were not different than the comparison group. Blood lead levels for all soldiers were within limits currently considered to represent no increased risk of adverse reproductive effect. Based upon system specifications and use restrictions, soldier operators who perform duties as directed should not be exposed to microwave radiations in access of limits currently accepted as safe. The results of this study should not be used to justify an immediate need to take emergency corrective interventions because they represent preliminary findings with limited statistical power. However, further studies incorporating larger numbers of subjects with potential exposure-related adverse reproductive lead and microwave exposure effects should be expeditiously coordinated and performed. Increased numbers of study participants in all groups will increase statistical power and should allow more optimal characterization of potential lead and microwave exposure effects on male fecundity.

NOTICE

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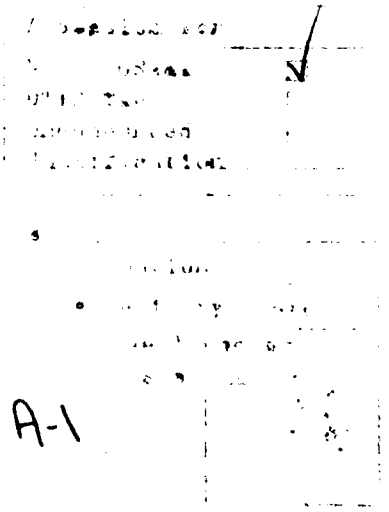

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PREFACE

Special recognition and appreciation is directed to the dedicated soldiers who participated in this study.

Collaborative scientific, statistical, and technical assistance was provided by the National Institute for Occupational Safety and Health (NIOSH). Dr. Janet Haartz, Director of Biomedical and Behavioral Science (NIOSH), and Lieutenant Colonel Joel Hiatt, USABRDL Executive Officer, negotiated and endorsed the memorandum of understanding.

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INTRODUCTION

Lead is among the earliest known metals identified and fashioned by man. It has been associated with a wide variety of social and industrial uses. The poisonous potential for lead in human populations has been recorded since ancient times. The spectrum of adverse health effects associated with lead exposure represents some of the earliest reported occupational exposure hazards. Both acute and chronic adverse effects have been reported from exposure t· lead. The plethora of symptomatology, clinical presentation, and laboratory confirmation possibilities has resulted in a substantial volume of scientific and medical literature.

The potential for lead exposures to cause adverse reproductive health effects in both males and females of reproductive age has been incorporated into the current legal occupational exposure standard. Promulgated by the Occupational and Safety Health Administration (OSHA), the "lead standard" requires special monitoring, administrative controls, biological monitoring, and counseling for all employees of reproductive age who are potentially exposed to lead in the workplace.

Requirements of the lead standard, 29 CFR 1910.1025, are directed toward employers and employees with a potential for workplace exposures to lead. The standard, promulgated in 1978, defines "lead" as metallic lead, all inorganic lead compounds, and organic lead soaps. It excludes other forms of organic lead. The current 8 hour time-weighted average (TWA) permissible exposure limit (PEL) for lead is 50 micrograms of lead per cubic meter of air (μg Pb/m^3 of air). Workplace exposures exceeding the "action level" of 30 μg Pb/m^3 of air or measured blood lead level (BLL) exceeding 40 micrograms of lead per 100 ml of whole blood (μg Pb/dL) serve as triggers to initiate legally mandated actions. The standard requires notification of both male and female employees with potential exposures to lead in the workplace concerning possible reproductive hazards of lead. It mandates that blood lead levels are to be maintained below 30 micrograms per 100 ml of whole blood for employees of reproductive age who anticipate pregnancy "in the near future." In addition to requirements for special training, employees are to be provided with pregnancy tests and laboratory evaluation of male fertility upon their request.

The current recommended exposure limit for inorganic lead published as a "Threshold Limit Value (TLV)" by the American Conference of Governmental Industrial Hygienists (ACGIH) is 0.15 milligrams of lead per cubic meter of air.

The divergent levels recommended by scientists and required by legal authorities suggested that a scientific feasibility to adopt a military unique exposure recommendation existed. While the development of a military unique exposure standard for lead

appeared attractive, inadequate health data were available upon which to base technical documentation.

Field evaluations characterizing lead exposure levels of soldiers associated with firing the 155 MM and 8-inch howitzers have been previously performed. Documented lead exposure profiles have been characterized by prominent peaks of short duration followed by prolonged periods with essentially no additional exposure. In separate studies, evaluations of lead exposures have been completed using artillery with damaged bore evacuators and have demonstrated high ambient lead levels. The known toxicity profile of lead and unusual patterns of potential exposure during military missions have limited the potential for reliable medical risk assessments.

As a result of the unique lead exposure profiles of military individuals associated with artillery fire, improved definition and identification of possible adverse health effects from lead exposure were considered desirable. Development and request for approval of a less stringent military unique lead exposure standard have been considered as an attractive training scenario alternative. Recommendations to relax guidelines for military occupational exposures could not be formulated, however, without documentation of the absence of adverse health effects in exposed soldiers.

Recent advances in laboratory technology have generated equipment and methodologies that can be transported to the workplace for assessment of male reproductive function. In an effort to evaluate the reproductive status of male artillerymen, an on site, state-of-the-art laboratory assessment was proposed and approved.

In association with the Argonne National Laboratory, an assessment was initiated to identify potential correlations between duration of service and possible cumulative impacts of lead. The null hypothesis that cumulative lead exposure would have no effect on male reproductive capability of 155 MM howitzer crewmen was tested based upon a carefully administered subject interview history and clinical laboratory analyses. Laboratory analyses included total bone lead, zinc protoporphyrin level, blood lead concentration, limited endocrine evaluation, complete blood count profile, and semen analysis.

This study was designed to investigate the potential for adverse reproductive effects related to ambient airborne lead exposures. The study was directed toward a population of male artillery crewmen with the possibility for significant exposures to ambient airborne lead. It was postulated that the potential male reproductive impact may be more sensitive to lead exposures than other demonstrable adverse health effects. It was further postulated that military unique exposure scenarios would result in no demonstrable adverse reproductive effects.

BACKGROUND

Lead appears to be one of the first metals used by man, possibly as early as 4000 BC.[1] The earliest known lead containing artifact, probably crafted in about 3000 BC, was found in the Temple of Osiris at Abydos and remains on display at the British Museum.[2] There is evidence that the Hebrew, Chinese, and Egyptian cultures used lead at least as early as 2000 BC.[1,2] Ores rich in lead content were mined by the Phoenicians from deposits in Spain by about 2000 BC.[1] Galena, the sulfide of lead, continues to represent the main mineral from which commercial lead is produced.

The environmental durability of lead has been demonstrated during recent excavations of the ancient water distribution and baths system of Rome which were constructed approximately 2000 years ago. Many ancient lead containing ornamental artifacts and bronze coins from ancient Greece and Rome contained 3 to 30 percent lead content and remain well preserved.[2] A number of architectural treasures with leaden roofs were constructed during the fifteenth and sixteenth centuries and remain preserved to the present day.[2]

Although the symptoms of lead poisoning were apparently recognized during ancient times, Aristotle (circa 370 BC) has been given credit for the first causal association of lead with symptoms.[1] Adverse human experiences from lead exposure were reported in early Rome. At that time, Pliny the Elder recorded health effects identified in slaves associated with pottery manufacture [3] and shipbuilding.[1] The early observations were focused and amplified by Ramazzini in the 18th century. In the early 19th century, Tanquerel des Planches provided the first "modern description" of lead poisoning.[1,3]

In the United States, Dr. Alice Hamilton provided the first thorough analysis of lead exposures associated with American industry and described effects on workers during the early 20th century.[4] Through her autobiography, she has provided invaluable insight into her earlier scientific job-related studies concerning the industrial circumstances of the day. The document reveals the political and socio-economic impacts of adverse lead-associated health effects identified within industry. The reactions to her studies by politicians, employees, and management are reviewed from her perspective.[3]

The potential spectrum of adverse health effects of lead absorption is broad. General descriptive toxicology studies and related human pathophysiologic responses associated with lead exposure and uptake have been widely studied and recently reviewed.[1,4,5,6] In addition to the readily apparent adverse toxicological signs and symptoms of lead absorption, Dr. Hamilton

noted that many subclinical manifestations may be associated with lead exposure.[4]

Descriptions of acute and chronic effects of lead absorption following exposures have been presented in a variety of the lay and scientific literature. Reviews of the signs, symptoms, and health effects are recorded in classical literature [3] and in current textbooks of medicine [1], scientific literature [5,6], and legal requirements for employee protection/education.[7]

Historical acute pathophysiological manifestations of lead absorption have included gastrointestinal (lead colic) and neurological disease manifestations. Absorption related to chronic exposures has been associated with numerous health responses including the once common lead line of the oral mucosa. In addition, significant adverse health effects have been associated with hematological, gastrointestinal, renal, neurological, and reproductive abnormalities. The full-blown clinical spectrum of either acute or chronic poisoning is rarely seen in modern times.[8] However, non-specific complaints such as fatigue, headache, sleep disturbance, stomach pain, and myalgia must be carefully evaluated as possible sentinel health events in workers with potential lead exposures.[8] The term sentinel health event (SHE) has been introduced by NIOSH. It is used to refer to the occurrence of any disease or other adverse health effect which may be seen as manifestation of occupational exposures to chemical substances.[9]

Dose-response curves associated with toxic responses to chronic lead absorption in humans have been published.[7,8] The International Labour Organisation (ILO) has adopted no-adverse-response levels which have been adapted from World Health Organization literature.[8] Documentation of the OSHA rationale behind determination of the established PEL, based upon available scientific data and discussion, is provided in the announcement of the final lead rule administrative decision.[10] Both organizations identify the earliest recognized adverse health response to lead absorption as an alteration within the bone marrow secondary to interference with heme synthesis.[11] Inhibition of the enzyme heme synthetase prevents appropriate incorporation of iron into protoporphyrin IX (PP) and, therefore, inhibits the formation of heme. As a result, red cells released from the marrow following lead induced inhibition would be expected to demonstrate an increased concentration of PP. Induced changes in levels of protoporphyrin can be monitored for the life span of the cell in circulation, and, thereby, may be used as an indication of chronic effects of past exposures.

The ILO [8] and OSHA [7] identify delta aminolevulinic acid dehydrogenase (ALA-D) as the most lead-sensitive enzyme system in the human. The ILO considers 5-10 μg Pb/dL of whole blood to be

the level below which no inhibition of the enzyme will occur in red blood cells. Both organizations adopt similar levels of 20 μg Pb/dL as the minimum level associated with increased serum protoporphyrin levels. The potential for chromosomal anomalies has been suggested at blood lead levels above 30 μg/dL by the ILO. The same level has been adopted by OSHA as the biological exposure index limit for individuals of reproductive age.[7] Decreased motor nerve conduction velocity may occur at levels above 40 μg/Dl [8] and diminished hemoglobin content may be seen at levels of 50 [7] or 60-80 μg/Dl.[8] Brain dysfunction may occur in the fetus or infant with levels as low as 30 [8] while it is usually not seen until levels of 50-60 μg/dL [7,8] in the adult. Impaired renal function has been reported above 60 [8] with paralysis and encephalopathy seen at levels above 80 μg/dL.[8] Although adverse reproductive findings have been identified in association with lead,[6,7,8,12] the relationship between exposure and effect remains poorly characterized.[13] Teratospermia has been reported with mean blood levels of 53 μg Pb/dL with hypospermia and asthenospermia at mean blood levels of 41 μg/dL.[7]

Documentation of the rationale for the recommendation of the Threshold Limit Value (TLV®) for ambient air-borne lead exposure has been provided by the American Conference of Governmental Industrial Hygienists (ACGIH).[14] Current scientific controversy and comparison with the OSHA requirement are reflected within the rationale. In contrast to the PEL of 50 micrograms of lead per cubic meter of air, the current TLV recommendation is a level of 0.15 milligrams (150 micrograms) of lead per cubic meter of air.

Adverse reproductive health effects following chronic lead exposures have been relatively recently reported and remain poorly defined. Based upon current documentation used by OSHA, it would appear that adverse effects in the male reproductive system could occur from chronic exposure with blood levels between 40 to 50 μg Pb/dL of blood.[6] The Occupational Safety and Health Administration requires medical removal of workers from lead exposure if blood lead levels exceed 40 μg/dL. In addition, OSHA requires that employees be informed of potential adverse reproductive effects and recommends a blood level below 30 μg/dL be maintained for both males and females who intend to have children.[7] NIOSH has drawn attention to the historical association of lead as a reproductive hazard and observed that data derived from modern workplaces have been conflicting.[13]

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Army Medical Department concerns about potential lead exposures were identified as a result of health hazard assessments related to several major military weapons systems. Potential exposures associated with performance of duties of artillery crewmen prompted the U.S. Army Surgeon General to initiate acute and chronic studies of selected crew members. Military evaluations initiated in 1985 identified high lead levels in combustion product emissions from the M109 howitzer when the bore evacuator was damaged.[15,16] Other analyses related to howitzer firing have demonstrated time-weighted air lead concentrations above the 8-hour OSHA Permissible Exposure Limit (PEL) of 50 μg Pb/m^3 of air.[17] The Argonne National Laboratory has provided documentation of the physical and chemical characterization of lead emissions from both the 8 inch and 155MM howitzers. At approximately the same time as the initial data for lead exposures were being characterized, the U.S. Army Materiel Command identified plans to double the amount of lead in high zone charges as a part of the Howitzer Improvement Program (HIP). In addition to lead aerosol characterizations, biological responses to lead exposures of soldiers were measured in the Operational Test of the Crew Ballistic Shelter. Additional lead aerosol related studies continue, performed under Interagency Agreement between U.S. Army Medical Research and Development Command and the U.S. Department of Energy (see Appendix A).

Relatively recent technological advances and scientific information have focused attention on use of semen as an indicator of exposure to reproductive hazards.[18,19,20] Semen analysis has been shown to be a useful indicator of male reproductive functional effects associated with occupational exposures to ethylene dibromide.[18] In the now classic and well publicized human study, an apparent decreased rate of fathering children stimulated male reproductive function evaluation in 1,2-dibromo-3-chloropropane (DBCP) exposed workers at a pesticide factory.[21] More than a decade previously, the detrimental effects of DBCP had been demonstrated in animal toxicology testing. In spite of those findings, decremental effects of workplace exposures upon male, human reproductive function were not anticipated. However, despite low concentrations of DBCP identified in sampling and analysis, a relationship between duration of exposure and degree of effect was demonstrated in exposed male workers. Exposure to DBCP was shown to result in oligospermia and azoospermia, associated with elevations of both follicle-stimulating and luteinizing hormones. Reduction in sperm motility and an increase in abnormal sperm forms were identified in individuals who were not azoospermic.

A diminished sperm count has been reported without endocrine dysfunction in lead-exposed men.[22] An earlier report had noted asthenospermia, hypospermia, and teratospermia in men with increased blood lead concentrations.[12,22]

Abnormal sperm motility has been reported to be associated with adverse effects in lead poisoned workers where lowered sperm counts and abnormal sperm forms were identified.[12] No previous efforts have been made to identify potential male reproductive effects in soldiers who have been exposed to lead aerosols as a result of weapons system use.

EXPOSURE CHARACTERIZATION

Artillery crewmen are distinguished by the 13B Military Occupational Specialty (MOS) identifier. As a result of career assignments, artillery crews are usually associated with operational assignments related to one type of weapon system.[23] Several types of exposure to weapons combustion products are possible, depending on the length of military duty, type of weapon system, and operational scenario. Soldiers may be exposed to weapons emissions during basic training, advanced individual training, materiels developmental testing, materiels operational testing, military unit training exercises, and armed combat. The highest potential exposures to lead aerosols are associated with firing of high lead-containing, high zone, or extended range, charges. High zone charges are uncommonly used in routine training exercises, but are a major source of potential lead exposure in field combat.[23] Excessive exposures to lead are not always associated with artillery firing. For example, one study performed to characterize weapons combustion products during a routine training exercise, failed to consistently identify exposures of soldiers to lead aerosols above the detection limit.[24]

Measurement of potential lead exposures was performed by collecting 20 samples during an operational test of two 155MM howitzers in 1985.[23] Two of the 8-hour TWA exposures were above the OSHA PEL of 50 $\mu g/m^3$ and three were above the 30 $\mu g/m^3$ of air action level. Data from fifteen minute grab samples indicated that transient peak exposure levels exceeded 1 mg Pb/m^3 of air. Although the types and numbers of expended rounds were not identified, the presence of high ambient lead exposure potentials during the firing events suggested that high zone charges were fired. The highest blood lead recorded was 33 $\mu g/dL$ of blood.

In a separate study of weapon combustion products, exposure evaluation was centered around three intensive 96 hour firing scenarios.[23] For the three exercises, all artillerymen exceeded the OSHA PEL when computed as a 24-hour TWA, and the 8-hour TWA was exceeded about 85 percent of the time. In the third firing scenario, 23 percent of individuals exceeded the 24-hour TWA by a factor of at least 6 times. Eight-hour TWA lead aerosol exposure concentrations were recorded between 3.96 and 61.9 μg Pb/m^3 of air. FEP and blood lead values increased with exposure. Twelve individuals were found to have blood lead levels between 30 and 40 $\mu g/dL$ of blood.

In the most comprehensive study, the quantitative and qualitative characteristics of lead aerosols generated by artillery firing using the 8 inch howitzer (M110) and three types of 155 MM Howitzer have been described.[17] The types of M109 evaluated were the M198 Field Piece, M109A3E1 (Cobra) production

design, and the upgraded M109A3E2 (Howitzer Improvement Program or HIP). An independent review and abbreviated summary of the draft report findings and analysis from that study are provided in the remainder of this section.

At least three potential contributing components of exposure of crewmen to airborne particulates were identified during artillery firing.[17] The visible and invisible gases expelled associated with explosion of the propellant cool, condense and coalesce to generate a particulate aerosol. The blast wave causes suspension of a secondary potential source of particulates from surface dust. After firing, a blue-white smoke flows from the muzzle and/or breech which contains suspended particulates produced from the blast.

Evaluation of the M110 howitzer equipped with a Crew Ballistic Shelter (CBS) was performed at Aberdeen Proving Ground in March 1987.[17] Characterizations of the aerosol contents, concentrations, and aerodynamic particulate sizes were based upon a total of 117 rounds fired over the four study days. Analysis of five separate rounds was performed in an effort to compare aerosols generated by different charges. Three of the individual round firing samples were obtained for Zone 7 and two for Zone 9 (high lead/high zone) propellant charges.

Use of lead is important as a de-coppering agent to control copper deposition inside the artillery tube as a result of firing. Lead is a component of some propellants, such as the M203 propellant, or may be used as a foil inserted into the breech with the charge before firing. Prior to firing, Zone 7 charges contain one ounce of lead carbonate. In comparison, Zone 8 charges with M203A1 propellant contain 156 grams (5.5 ounces) of lead while Zone 9 charges have 10.25 ounces of lead foil per charge sewn into the propellant bag.[17,23]

Evaluations of combustion products from firings of the M109A3 were performed using the M109A3E2 Howitzer at Yuma Proving Ground (YPG) in August 1987.[17] Aerosol samples were collected and analyzed from five locations inside the weapon cab and five were obtained in nearby outside positions. One year later, aerosols generated by firing the M109 Field Piece, Cobra, and HIP (M109A3E2) at YPG were sampled and analyzed. An important variable which could result in quantitative differences between the two M109 systems is that the crew compartment cab (turret) is much larger in the HIP weapon system design.

Quantitative and qualitative characteristics of the lead aerosols differed significantly between the blasts from two types of 8 inch zone charges and the 155 MM howitzers.[17] Lead particulates in the CBS of the M110 appear to be significantly larger than the particulates found within the cab of the M109.

Approximately 32 percent of the particulates in the CBS were less than 2.5 microns (μm) in size, compared to the cab of the M109 where 83-93 percent of the aerosol components were less than 3 μm and 81 to 86 percent were less than 0.3 μms.

Lead aerosol particulates from the M110 blasts were spherical in shape and were found to be between 0.5 and 10 μms in size, with the majority below 5 μms. Particulates between 5 and 20 micrometers in size were irregular in shape and contained very limited amounts of lead. The majority of particulates in the large size range (i.e. 5 - 20 μms) were composed of aluminates and aluminosilicates. Lead particulates from the high zone charges were of respirable size, i.e. 5μm or less. 32 percent of lead particulates aerosols generated from firing high zone charges were less than 2.5 μms in size. Particulates between 2.5 and 5 μms represented the remaining 68 percent of lead aerosol from the high zone charges. Breech aerosols contained 3 to 6 percent lead by weight for high zone charges.

Aerosols obtained after firing the M109A3E1 and M109A3E2 contained 80 to 85 percent of the total lead content in particulates less than 0.3 μms in size. Suspended particulate levels were elevated for longer durations following M109 firings than following M110 firings. Larger, spherical particles associated with M109 firings were not composed of lead but were found to be coated with tiny lead particulates. Breech aerosols generally were composed of 3 - 6 percent lead while muzzle aerosols typically were composed of 15 - 20 percent lead. In one case, the muzzle blast zone aerosol was found to be 22 percent lead by weight. Less than one percent of breech or muzzle aerosol particulates were present as unidentified organic lead compounds. Two lead-containing compounds were tentatively identified, but not validated, on separate occasions: potassium lead carbonate hydroxide and ammonium lead copper nitrate.

Concentrations of airborne lead aerosols measured inside and outside crew cabins after firings of both the M109 and M110 were dependent upon a number of variables.[17] Differences were found to be associated with type of weapon system, tube elevation, type of charge, wind direction/speed, time duration between firing and breech opening, presence of a bore evacuator, presence/position of hatches, position of the muzzle brake, and air sampling instrument location. As an example, concentrations of lead were approximately tenfold higher when the firing elevation was compared between the loading position elevation and high elevation of field firing. Another possible variable that could result in differences of exposure between the two weapons systems is the design angle position of the muzzle brake. The 8-inch Howitzer has a muzzle brake which deflects the blast at 90 degrees from both sides of the tube while the brake of the 155mm Howitzer is directed rearward at 45 degrees on both sides of the barrel.

Air lead concentrations were low (1-2 μg Pb/m^3 of air) when high lead charges were fired from the M110 associated with a tailwind. In contrast, airborne lead aerosol concentrations were very high (600 μg Pb/m^3 of air) at all crewmember positions when high zone charges were fired with the M110. Calculations based upon the quantitative collection of samples demonstrated that most of the ten ounces of lead foil used in the high zone charges was recovered as lead aerosols of respirable aerodynamic size. Airborne copper levels were found to range between 1 and 230 μg of copper per cubic meter of air. Copper/lead ratios were identified in the range between 0.3 and 0.7 and were felt to demonstrate the effective de-coppering role of lead.

Environmental air concentrations of lead in surrounding areas of both the M110 and M109A3 weapon systems were high in all charges that utilized high lead containing propellants or lead foils. Ambient values in the near vicinity of the M110 was 309 to 381 μg Pb/m^3 of air compared to ranges of 109 to 496 for the M109A3E1 and 427 to 627 μg Pb/m^3 for the HIP weapon system. It has been suggested that all lead was aerosolized with the blast and condensed upon cooling to generate muzzle blast aerosols.[17] Those aerosols appeared to flow into the cab through the breech if it was opened shortly after firing. Total lead mass of aerosols at the muzzle blast zone were similar for the M109A3E1 (18.5 percent lead by weight) and M109A3E2 (17.5 percent by weight).

Air lead exposure to crew members of the 8-inch howitzer blast aerosol components was measured using breathing zone samplers.[17] Twenty four-hour TWA exposures of crew members firing the M110 with low zone charges in weapon systems without the crew ballistic shelter were between 0.1 and 0.4 μg Pb/m^3 of air. Twenty four-hour TWAs for crewmembers firing the M110 with high zone charges in weapons with the crew ballistic shelter were between 2 and 30 μg Pb/m^3 of air. Mean exposures for two groups of crew members were 3 and 11 μg Pb/m^3 of air. Maximum exposures were associated with a 1-5 knot headwind, following a 12-hour firing scenario. The 12-hour TWA for that day was 60 μg Pb/m^3 of air, substantially in excess of the acceptable 12-hour TWA for lead.

In one M109 firing scenario, airborne lead concentrations were measured between 100 and 200 $\mu g/m^3$ of air at all crew positions inside the cab.[17] In contrast, muzzle blast emissions contained between 150 and 600 μg Pb/m^3 of air. Ambient lead aerosol concentrations in the muzzle blast area were consistently higher than those measured at crew member locations inside the cab. Airborne copper levels ranged between 25 and 100 μg of copper per cubic meter of air at crew member positions, with copper to lead ratios in the range of 0.2 and 0.7. Copper levels

in the muzzle blast aerosol was as high as 250 $\mu g/m^3$ of air. Airborne copper levels within and outside the weapons cabs were identified in the range between 1 and 365 $\mu g/m^3$ of air. These levels were below ACGIH recommended exposure level of 1 milligram of copper dust per cubic meter of air but were at times higher than the NIOSH recommended exposure level of 100 micrograms of copper fume per cubic meter of air.

In another series of measurements conducted over a 3-day period, air inside the cab of the HIP had a mean TWA of 142 μg Pb/m^3 of air.[17] Concentrations of lead in outside air, within the blast zone, ranged from 427 to 627 $\mu g/m^3$ of air. Downwind air samples contained 76-94 μg Pb/m^3 of air at 14 meters and 22-30 μg Pb/m^3 of air at 41 meters from the weapon. No lead was detected upwind at 14 meters from the weapon.

Airborne lead concentrations inside the M110 with the crew ballistic shelter were much more variable and dependent on wind conditions than those measured inside the M109A3 with cab.[17] Variability of exposures were greater for crew members in the M110 with the CBS than for crew members of the M109 howitzer with cab. At the gunner position, exposures ranged from 1 to 603 μg Pb/m^3 of air depending upon the wind direction. In contrast, airborne lead exposures at the gunner position in the cab of the M109 varied between 145 and 180 with a tail wind to a maximum of 205 μg Pb/m^3 of air with a head wind. Although no clear reasons for the differences were apparent, the possibility that the open CBS afforded a different exposure profile was offered. In an average for both M109 systems, the mean content of lead at the gunner position was 5.3 percent by weight compared to 18.5 percent in the muzzle blast zone.

To document airborne lead exposures, breathing zone samples were collected for soldiers associated with firing the M110 with CBS at Fort Sill.[17] The length of firing period was compared to the air sampling duration and the actual exposures were adjusted to reflect exposures in terms of the real-time firing durations. The value of 60 μg Pb/m^3 of air was calculated to represent the 12-hour TWA for crew members associated with firing Zone 9 charges through the M110 with CBS at a rate of 20 rounds per hour. The value of 60 was approximately one tenth of the previously measured exposure concentration for the same weapon system fired at a lower tube elevation at Aberdeen Proving Ground (603 $\mu g/m^3$ of air). The five crew members who spent the longest time actively firing demonstrated a mean 28-hour TWA of 31 $\pm$ 9 (mean $\pm$ SE) micrograms (μ) per cubic meter of air. The crew member with the highest documented exposure had a 28-hour TWA breathing zone sample of 60 μPb per cubic meter of air. However, the actual firing time was only 6 hours over the 28-hour period. For the five crew members, averaged, the actual 6-hour time-adjusted airborne lead concentration was 140 μg Pb/m^3 compared with the maximum adjusted concentration of 270 μg Pb/m^3.

Biological responses associated with airborne lead exposures were identified and reported within the group of soldier subject volunteers at Fort Sill.[17] Two groups of soldiers were evaluated. Biological and medical surveillance markers were studied in crew members who first fired low zone, low lead charges then completed a two week training course. After training, soldiers fired both low zone and M188A1 Zone 9 (high lead) charges from the M110A2 with a crew ballistic shelter. Each of the M188A charges contained 10.25 ounces of lead foil which was available to volatilize during firing to form a lead-rich aerosol cloud. In an effort to characterize exposure of crew members from lead aerosols, soldier participants wore breathing zone air sampling devices during the firing periods.

Blood and urine sample collection were performed with questionnaire administration and nerve conduction velocity measurement at the firing location, on the first Monday after the firing period. The same tests were repeated eleven weeks after the firing exercise. Several statistically significant biological alterations which were identified. The changes included rises in blood lead levels, rises in protoporphyrin levels, slight decreases in hematocrit, and decreases in nerve conduction velocities of selected nerves. No adverse health symptoms or signs were reported to be associated with the exposure.

Crew members of one of the two groups fired a total of 984 high lead zone 9 charges. The mean rise in blood lead levels within the group was 1 μg Pb/dL of blood. Eight weeks post exposure, the mean blood lead was slightly decreased (0.9 μg/dL) from the established mean baseline level. In contrast, the second group of soldiers who fired a total of 2379 Zone 9 (high lead) charges using the M110 with CBS were found to have higher airborne lead exposures with concomitant increases in blood lead values. In this group the mean blood lead levels increased from 4.9 to 11.4 μg Pb/dL of blood. When using each soldier subject as his own control, the mean increase of blood lead was found to be 6.9 micrograms per deciliter of blood. The increase in blood lead levels was sustained in the group with maximum exposure for at least 7 weeks, although blood lead levels were seen to plateau during the first 2 weeks despite ongoing exposure. The number of high zone discharges and the prevailing wind direction/speed were found to affect exposure and the potential to generate changes in blood lead levels.

Biological data were obtained and compared between two different crews; the 4/4 Field Artillery (4/4 FA) had relatively little lead exposure (1.4-6.2 $\mu g/m^3$ of air for 22 hour exposure time) compared to the 2/18 Field Artillery (2/18 FA) with higher exposures (1.0-27.1 $\mu g/m^3$ of air for 25 hour exposure time).

Each crew member served as his own control. Increased exposures to lead were associated with statistically significant elevations of blood lead and free erythrocyte protoporphyrin levels. Blood leads ranged between 3 to 9 μg /dL for pre-exposure and 5 to 14 μg/dL for postexposure in the 4/4 FA group. For the 2/18 FA, blood lead levels were from 3 to 7 μg/dL for pre-exposure to between 7 and 17 μg/dL postexposure. In addition, increased lead exposures were associated with depression of hematocrit and a "borderline" delay in nerve conduction. Results, adapted from Bhattacharyya et.al.,[17] are summarized in Table 1.

Table 1. Mean changes in blood lead (BLL in μg/dL blood), hematocrit (Hct in %), and free erythrocyte protoporphyrin (FEP in μg/dL blood) [17]

Parameter	Group	Baseline to Immediate post fire	Immediate post fire to six weeks after fire	Baseline to six weeks after fire
BLL	4/4 FA	1.0 ± 0.5	- 1.9 ± 0.5*	- 0.9 ± 0.3*
	2/18 FA	6.9 ± 0.7*	- 1.2 ± 0.6	5.4 ± 0.5*
Hct	4/4 FA	0.43 ± 0.58	1.46 ± 0.78*	1.50 ± 0.41*
	2/18 FA	- 1.47 ± 0.63	[illegible] ± 0.40*	0.12 ± 0.55
FEP	4/4 FA	- 0.31 ± 0.72	1.42 ± 1.06	1.30 ± 0.81
	2/18 FA	2.22 ± 0.65*	- 5.45 ± 0.62*	- 2.67 ± 1.18*

* significantly different than zero ($p<0.05$) using the Student's 2-sided t-test for paired observations.

There are a number of models that can be applied to 8-hour exposure levels in order to develop potentially acceptable exposure limits for unusual work schedules.[25] The General Duty Clause of the OSHA Act permits citation of employers who allow employees who work extended shifts without adjustment of allowable exposure limits. The first recommendation concerning modification of exposure limits was published by Brief and Scala in 1975.[25] This model accounts for both an increased exposure time and diminished recovery time from cumulative workplace exposures. The concept stresses that the peak internal tissue levels may be higher during extended exposure times than during normal work cycles. In the description of the model, there is a disclaimer to the use of this model for novel work schedules that involve 24-hour continuous exposures.

The Brief and Scala reduction factor over-estimates exposure and is calculated independently from toxicant half-life. The reduction factor formula during novel work shifts is as follows:

$$\text{TLV reduction factor} = (8/h) \times (24-h)/16$$

where h is the number of hours exposed per day. For example, if soldiers were exposed to lead aerosols over a 12 hour exposure period, the reduction factor would be as follows:

$$\text{TLV reduction factor} = (8/12) \times (24-12)/16$$

$$= 0.49$$

The adjusted TLV would then be calculated as follows:

$$\text{New TLV} = RF \times 50\ \mu g/m^3$$

$$= 0.49 \times 50\ \mu g/m^3$$

$$= 24.5\ \mu g/m^3$$

When used to compensate for novel exposures during the 7-day, "40-hour adjusted" workweek for periods of continuous exposure less than 24 hours, the reduction factor formula is as follows:

$$\text{TLV reduction factor} = (40/h)(168-h)/128$$

where h is the number of hours exposed per week. As an example, using this formula, the "adjusted" TLV for 4 consecutive days, 22 hours each day calculated exposure concentration of lead would be:

$$\text{TLV reduction factor} = (40/88)(168-88)/128$$

$$= (0.45)(0.62) = 0.27$$

Therefore the "new TLV", using the Brief and Scala model for lead would be:

$$50\ \mu g\ Pb/m^3 \times 0.27 = 13.5\ \mu g\ Pb\ /m^3.$$

Another model which has been recommended for calculation of an adjusted exposure is the OSHA model.[25] For comparison with the Brief and Scala model, the "equivalent PEL" based upon a 88-hour exposure in a 7-day workweek as computed using the OSHA adjustment would be:

$$\text{Equivalent PEL} =$$

$$\text{8-hour PEL} \times \text{40 hours/hours of exposure per week}$$

Therefore, for an 88 hour exposure scenario using the OSHA model, the equivalent PEL would be:

$$= 50\ \mu g/m^3 \times 40/88$$

$$= 22.7\ \mu g/m^3 \text{ of air}$$

The potential for high exposures to airborne lead was identified in the updated health hazard assessment report (HHAR) on the HIP.[15] A concern that blood lead levels of soldiers firing howitzers may reach levels of 30 to 40 μg Pb/dL of blood was expressed. At those blood lead levels, the potential for spontaneous abortion or sterility was identified. A 24-hour PEL of 16.7 $\mu g/m^3$ of air, i.e 50 $\mu g/m^3$ X 8 hr/24 hr, was identified in the HHAR. An action level of 10 $\mu g/m^3$ was recommended to initiate implementation of engineering control, personal protection, or administrative restriction.

Statistically significant differences in lead exposures of soldiers were documented between the HIP (mean air lead of 22.5 $\mu g/m^3$) and the M109A3 (mean air lead concentration of 40.9 $\mu g/m^3$). Both values exceeded the applicable PEL.[15] The highest average blood lead was 22 μg/dL for HIP crewmembers and 24.6 for crewmembers of the M109A3. Of 13 blood lead levels above 30 μg/dL, three were observed in HIP crewmembers and the remaining 10 were identified in M109A3 crewmembers.

Analysis of data for a 96-hour scenario demonstrated that the PEL in a training environment would be exceeded for any soldier if more than 13 of the M119 rounds were fired in a 24-hour period.[15] The necessity for appropriate personal protective equipment (e.g. respiratory mask), worker training, and medical monitoring was identified for soldiers routinely exposed above the action level in a training environment.

The bore evacuator of the M109 could be damaged in a training or field combat scenario resulting in an increased release of combustion gases into the crew compartment associated with breech opening. In a special study, measurements were performed inside the CBS of the M109A1 and M109A3 to identify the possible airborne exposures to lead aerosols if the bore evacuator was damaged.[26] Airborne lead levels in the CBS of the M109A1 with a damaged bore evacuator ranged from 0.025 to 1.97 mg Pb/m^3 of air. Comparative values in the CBS of the M109A3 ranged from 0.008 to 6.10 mg Pb/m^3 of air.

MATERIALS AND METHODS

This present study was performed in association with the technical proposal for modification of an interagency agreement between the U.S. Army Medical Research and Development Command and U. S. Department of Energy. The proposal, entitled "Lead Exposure and Biological Responses in Military Weapon Systems" is enclosed as Appendix A. The study, U.S. Army Project Order No. 86PP6821, was performed by the Argonne National Laboratory. Authority was based upon the identified requirement for additional information upon which to develop the final health hazard assessment of artillery weapons systems.[15]

All aspects of the present study were reviewed and approved by the Department of the Army Surgeon General's Human Subjects Research Review Committee. The study progress was monitored by the Human Subjects Research Review Committee.

Volunteer subject participants were initially solicited from members of active self-propelled 155MM Howitzer crews and a pre-established Argonne National Laboratory "control" group. The Argonne study was performed to evaluate lead deposition and possible effect in artillerymen as a function of duration of service. Criteria for subjects of the control group of soldiers were selected by the Argonne principal investigator. In the protocol design for the reproductive study, the Argonne associated "controls" were accepted and expected to reasonably represent the reproductive status of the "non-lead exposed" population.

In order to solicit participation of study subjects, the purpose and design of the study were presented to groups of prospective subject volunteers. After complete description of the study, informed consent from the volunteers was documented. A volunteer agreement affidavit (DA Form 5303-R) was completed by the investigator and signed by the each of the volunteers prior to initiating the study. A copy of the consent form was provided to each subject and a copy was placed in his outpatient medical record.

All study subjects agreed to provide historical information by interactive questionnaire, a blood sample, and a semen specimen. Blood samples were collected following semen sample presentation for laboratory analysis. Successful completion of the questionnaire, provision of a blood sample, and presentation of a semen specimen were required for retention as a study subject. Vasectomy was not accepted as an exclusion criterion.

Although a minimum of 25 lead-exposed subjects and 25 control subjects was initially considered acceptable, every attempt was made to obtain as many study subjects as possible.

Volunteer participants were assigned a randomly generated numeric identifier to facilitate blinded laboratory analysis. In addition, appointment times for submission of semen analysis and participation in subject interview were randomly scheduled.

All study participants completed a standardized, validated, interviewer administered questionnaire. The questionnaire had been previously developed and partially validated by NIOSH as an effort to identify potential exposures to reproductive hazards and past or present reproductive difficulties. Copies of the participant and non-participant questionnaires are provided at appendix B and C, respectively. Approximately 95 percent of the questionnaires were administered by the author. The remaining 5 percent were administered by a trained assistant. As a result of abbreviated timelines and logistical limitations, non-participant questionnaires were not administered to those individuals who chose not to participate following solicitation, i.e. true non-participants. In contrast, information requested in the non-participant questionnaire was solicited from soldiers who were enrolled in an independent study of soldier demographics. Non-participant questionnaires were distributed by the principal investigator of the demographics study for self-administration by subject volunteers and returned for analysis in this study.

By the third day of the study, it was apparent from subject interviews that the Argonne Laboratory "control" population had been drawn from the military intelligence occupational specialty. Many soldiers in this specialty perform duties associated with the use of active microwave transmitter/receivers or passive microwave receivers. As a result, many of the study pre-selected "control" subjects provided a positive history of potential work related microwave radiation exposure. While these subjects met every definition of control subjects in the Argonne study, the positive potential microwave interview responses were troublesome with respect to the reproductive function study. The potential for microwave exposure seemed unacceptable as a criterion for "true control" subjects in the reproductive study. Since the frequent use of potential sources of microwave radiation was unanticipated in the study design, complex statistical analysis for interaction or confounding impacts appeared essential.

On the third study day, Dr. Schrader related his independent observation that an unusual frequency of low sperm counts was being recorded. At that time, he was informed of the unexpected observation among "control group subjects" concerning historical potential for microwave exposure as a significant part of their military occupational duties. After discussion, it was determined that theoretically possible adverse reproductive effects from potential microwave exposure precluded acceptance of the Argonne controls as a reference "normal" reproductive population. At that time, it was further decided that the preferable alternative was active solicitation of additional

study subjects. In addition to the possibility of microwave exposure, the factor of elevated environmental temperature represented a possible adverse influence on male reproductive function. As a result, control participants were solicited from military occupational specialties with similar environmental temperature exposure profiles but without lead or microwave exposures.

For purposes of the present study, individuals who participated only in annual small arms qualification firing were not considered to have a cumulative risk of lead exposure or retention.

Blood pressure, nerve conduction velocity, blood lead, and bone lead concentrations were performed by the staff of the Argonne National Laboratory (Appendix A) and will be separately reported. This reproductive study protocol amended the basic Argonne protocol by substitution of a separate questionnaire and addition of several laboratory analyses. Clinical laboratory analysis performed in association with the reproductive protocol included zinc protoporphyrin level, complete blood count and profile, reproductive hormone profile, and semen analysis.

Zinc protoporphyrin (ZPP) level was obtained by withdrawal of 15 milliliters (ml) of blood by antecubital venipuncture and submission to Brooke Army Medical Center for analysis. All ZPP analyses were performed in an accredited clinical laboratory facility.

A Complete Blood Count (CBC) was performed after withdrawal of 15 ml of blood which was then submitted for analysis of total count by Coulter Counter, manual differential white blood cell count, and blood smear. CBC analyses were performed in an accredited clinical laboratory at the Fort Hood medical treatment facility.

Reproductive hormone analyses were performed after withdrawal of 15 ml of blood which was sent to NIOSH for hormone level determinations. Endocrine profile analysis was performed for blood levels of follicle stimulating hormone, luteinizing hormone, testosterone, estradiol, and prolactin.

Total and free testosterone concentrations in blood serum were determined using commercial radioimmunoassay (Coat-A-Count®; Diagnostic Products Corporation, Los Angeles, CA.; Cat. Nos. TKTT2 & TKTF2). Serum concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), and prolactin were determined using commercial two-site time-resolved fluoroimmunometric assays (Delfia®; Wallac Oy, Turku, Finland; Cat. Nos. 1244-031, 1244-017, & 1244-018). Both monoclonal antibodies within the LH assay are directed against the β subunit; the two antibodies for the FSH assay are directed against the α and β subunits, respectively. Sera pools with low,

medium, and high amounts of each analyte (Lyphochek®; Bio-Rad Labs, Anaheim, CA.; Cat. No. C-370-5) were distributed throughout all assays. Intra-assay coefficients of variation were: 4.3% for total testosterone; 9.2% for free testosterone; 4.2% for LH; 3.9% for FSH; and 5.3% for prolactin. Inter-assay coefficients of variation were 1.8% for total testosterone and 6.2% for free testosterone.

Semen analysis was conducted according to the methodologies endorsed by the consensus workshop of andrologists conducting human reproductive toxicology field studies.[27] Exact instructions were provided to each man to ensure the semen sample was collected by masturbation after a set time of abstinence (usually 2 days), and delivered to the laboratory within 1 hour from the time of ejaculation. A NIOSH produced video tape with these instructions was shown to all participants of the semen study.[28] At the time of collecting the semen sample, each subject recorded the duration of abstinence, time of semen collection, and any information regarding spillage. A label on each jar facilitated the recording of this information.

Within 1 hour of ejaculation, semen was submitted to the Fort Hood Preventive Medicine Clinic for on-site analysis by collaborating NIOSH scientists. Temperature, osmolarity, motility, sperm count, viability analysis, pH, and sperm morphology/morphometry were performed by Dr. Schrader and Mr. Turner. A video tape recording of each fresh preparation microscopic specimen was made at Fort Hood for later computerized analysis by the collaborating NIOSH scientists upon their return to Cincinnati. Separate analyses of glycerylphosphorylcholine level, zinc, and fructose levels in semen and a sperm function (penetration) study were performed at NIOSH. In addition, when the semen volume of the submitted specimen was adequate, a specimen was provided for DNA stability assay at NIOSH.

Semen analyses were conducted in two phases. The initial evaluation of the sample was conducted when the sample arrived at the field site laboratory, and consisted of recording the temperature, turbidity, color, liquefaction time, volume, osmolality, and pH of the semen. Video recordings for motility assessments, viability estimates, sperm counts, preparation of microscopic slides, and preservation of seminal plasma was also conducted at that time. Morphologic and morphometric analyses of sperm on slides and motility and velocity analyses of the sperm recorded on video tape records were performed later at NIOSH using a computer assisted sperm analysis system [CASA] (CellSoft®, Cryo Resources, New York).

Sperm viability was determined by two methods, Eosin Y stain exclusion [29] and hypoosmotic swelling (HOS assay).[30] These techniques test, respectively, for the structural and functional

integrity of the cell membrane.[31] Sperm concentration was performed in a Makler chamber and motility characteristics were measured in a MicroCell® chamber 20 μm deep in order for the sperm to move freely in all planes.

Measurements of sperm motility and velocity were conducted using a microscope stage warmed to 37°C. The CASA system, used for motility and velocity estimates, required the number of sperm per field to be reduced to minimize cell collisions. In order to optimize analysis using the 20 μm deep chamber, the sperm concentration should be less than 40 million/ml. Prior to analysis, semen samples were diluted 1:1 using tyrodes buffer. If this dilution failed to reduce the sperm concentration below 40 million/ml, an additional dilution using the same buffer was performed. Sperm motility parameters measured by the CASA system include curvilinear velocity (VCL), straight-line velocity (VSL), their ratio [VCL/VSL * 10] (LIN), amplitude of lateral head displacement (ALH) and beat cross frequency (BCF).

Sperm morphology was estimated on air dried, stained semen smears according to the method of Zaneveld and Polakoski.[32] Morphometric measurements of sperm were also conducted using the same slides according to methods of Schrader et. al.[33] Sperm morphology measurements included width, length, area and perimeter of the sperm head. Calculations for numeric comparisons of sperm shape included the ratio of width/length and the oval factor.

The oval factor was calculated using the formula

$$\text{Oval Factor} = 4\pi(\text{area})/\text{perimeter}^2.$$

Volumetric, pH, and physical characteristics of the seminal plasma were also analyzed using accepted methodologies.[34] Semen volume was determined to the nearest 0.1 ml using a plastic syringe. The pH was measured within 90 minutes of ejaculation using a gel filled plastic pH probe (Beckman Instruments, Fullerton, CA). Osmolality was determined with freezing point depression osmometer (model 3 MO, Advanced Micro Osmometer; Needham Heights, MA) within 90 minutes of ejaculation.

After performing the initial measurements, the semen was centrifuged at 3 x g for 10 minutes and the seminal plasma was removed and frozen at -60°C. The frozen specimens were stored until the biochemical analyses were performed. After thawing, Glycerylphosphorylcholine (GPC) was determined using the methods of Perez-Pelaez et al. [35] using a Perkin Elmer (model 554) spectrophotometer. Fructose levels were measured based upon the methods reported by Mann.[36] Zinc concentration was determined using the methodology of Fuentes et. al.[37] Fructose and zinc colorimetric assays were conducted using a Bausch and Lomb spectrophotometer (model Spectronic 83).

Two investigational methods, the sperm penetration assay (SPA) and the DNA stability assay, were evaluated to assess the possibility of identifying effects of toxicants on the male reproductive system. The sperm penetration assay, also known as the zona free hamster egg penetration assay, provides information on sperm function. The sperm were capacitated and then incubated with hamster eggs with the zona pellucida removed.[38] The frequency of sperm penetration was then calculated. Overnight capacitation of sperm occurred as the samples were transported to the NIOSH laboratory using an overnight mail delivery service.

The DNA stability assay provides information regarding genetic damage to sperm. To perform the assay, sperm DNA was stressed chemically and then stained with acridine orange. Double stranded DNA is stained green while single stranded DNA is stained red (very little if any RNA is present in mature sperm).[39] DNA stability analysis was performed with a flow cytometer.

RESULTS AND STATISTICAL ANALYSIS

All historical information gathered through administration of the questionnaire was collated, entered into a computerized database, analyzed, and used to categorize the description of the study population. A list of ***a priori*** covariate parameters, with abbreviations and descriptions, is provided in Table 2. Questionnaire data were used to assess the self-reported potential exposures to lead, microwave, and other physical or chemical interactions that could potentially interfere with sperm production or maturation. The non-participant questionnaire was not useful in the characterization of the true study non-participants, i.e. those individuals who were afforded an opportunity but elected not to participate in the study. Instead, the non-participant questionnaire was considered to have a limited utility for definition of a non-randomly selected population of soldiers representative of the "general population of soldiers."

Statistical Significance.

The calculated statistical levels of significance for comparative analyses are provided numerically in the tables of this section of the report. A focused discussion or brief notation of the statistical interpretation is entered in proximity to the specific table of the study variable under consideration.

Specific statistical numerical data in the tables are not highlighted with reference to accepted "significance" within the body of the tables. In this report, the value of "$p < 0.05$" was accepted as statistically significant for any single, specific variable that has been analyzed. Because of the large number of variables evaluated in this study, it was recognized that chance, alone, could occasionally result in findings of "significance". The possibility of this chance occurrence associated with the large number of study variables has been recognized and statistical calculations may be adjusted using the concept of Bonferroni inequalities.[40]

In the most conservative statistical comparison, analysis of the possible interactions of primary variables on the statistical significance on the specific parameter is recognized by use of the Bonferroni correction. If the Bonferroni correction is applied in the statistical analysis of a parameter, the "p value" for the specific parameter becomes

$$p = 1 - \alpha/n',$$

where α is 0.05 and "n'" is the number of dependent variables studied.[40]

Table 2. **A priori** covariate abbreviations and descriptions

---

ABS (Number of days abstained)

ALCOHOL (Amount of alcohol consumed at one time)
    How many bottles of beer
    How many glasses of wine
    How many shots (ounces) of liquor

CAFFEINE (Amount of caffeine consumed on an average day)
    Cups of caffeinated coffee
    Cups of caffeinated tea
    Bottles or cans of caffeinated cola or soda

FEVER (Viral or bacterial infections or flu that caused fever in the last 3 months)

HOTBATH (Frequency of very hot baths, saunas, or steam baths)
    "NEVER", "1-3/MONTH", "1-3/WEEK", "4+/WEEK"

MANCT (Sperm count, millions per ml)

MARIJ (Surrogate measure of marijuana use)
    How many [of 3 good friends] smoke marijuana or hashish regularly? Note: an answer of 2 or 3 is considered a surrogate measure of marijuana use.

RACE (Race/ethnic heritage)
    "White", "Black", "Hispanic", "Asian"

SAGE (Sample age in minutes)

SMOKE (Amount of smoking per day)
    # cigarettes per day
    # cigars per day
    # pipefuls of tobacco per day
    # units snuff/chewing tobacco per day

SPILLAGE

TYPNDRWR (Type of underwear worn)
    "Boxer shorts", "Briefs", "None", "DK/NA"

VENDIS (Diagnosed as having a venereal disease)
    Non-specific urethritis or discharge from the penis OR
    Chlamydia OR Syphilis OR Gonorrhea OR Genital Herpes

Therefore, in this study where a list of fifteen primary scientific variables (Table 3) was empirically accepted, the correction is

$$p = 1 - 0.05/15.$$

If the Bonferroni correction is used for any parameter in the study identified as a primary variable, the level of $p = 0.0033$ may be used as the most conservative level for acceptance as a level of significance, rather than the more customary $p < 0.05$.

In clinical cases where laboratory analysis is provided as an adjunct to the assessment of male fecundity, a total of five parameters are usually considered to be of primary importance. They are sperm count, percent motile sperm, percent viable sperm, percent normal sperm, and sperm morphology. If primary variables are limited to the five usually performed, the statistical Bonferroni correction for analysis of the "p value" would be

$$p = 1 - 0.05/5$$

For statistical analysis in this study, all tests were conducted at alpha = 0.05. In this study, then, the Bonferroni adjustment for multiple comparisons was applied to the identified primary variables by dividing the alpha level by the number of primary variables [40] prior to interpretation of the p value. Therefore, the adjusted p-value was declared significant if it was smaller than .05/15 = 0.0033. Significant changes in any of these variables after the Bonferroni adjustment (p-values smaller than 0.0033) should be taken as strong evidence of adverse effect due to lead or microwave exposure. Changes that were significant before but insignificant after Bonferroni adjustment (p-values between 0.0036 and 0.05) could also represent strong evidence of an adverse effect due to lead or microwave exposure. For these variables, however, a qualifying statement was added that the effect seen might be due to the large number of variables studied.

Changes not significant at alpha = 0.05 were considered as evidence of no adverse effect. Nevertheless, if some comparisons were close to significance, it was accepted as evidence for need of further study, dependent upon the context of the effects.

Table 3. List of primary variables with **a priori** potential covariates.

Primary variables	Potential covariates
1. MANCT (Sperm count per ml)	ABS, HOTBATH, ALCOHOL, SMOKE, TYPNDRWR, FEVER, VENDIS, SPILLAGE, MARIJ
2. NORMAL (Percent normal sperm)	HOTBATH, ALCOHOL, SMOKE, TYPNDRWR, FEVER, VENDIS
3. PCTMOT (Percent motile sperm)	TEMP, SAGE, MARIJ, CAFFEINE, SPILLAGE,
4. LVEL (Average linear velocity)	same as above
5. RATIO (Length/width)	ABS, HOTBATH, ALCOHOL, SMOKE, TYPNDRWR, FEVER, VENDIS, RACE
6. LH (Luteinizing hormone)	no confounders
7. TESTFREE (Free testosterone)	no confounders
8. TOTCT (Sperm count per ejaculate)	same as above
9. LENGTH (Average length of sperm head)	ABS, HOTBATH, ALCOHOL, SMOKE, TYPNDRWR, FEVER, VENDIS, RACE
10. WIDTH (Average width of sperm head)	same as above
11. AREA (Average area of sperm head)	same as above
12. PERIM (Average perimeter of sperm head)	same as above
13. PCTVITAL (Percent vital stain)	TEMP, SAGE, CAFFEINE
14. PCTSWOL (Percent swollen sperm)	same as above
15. PCTPEN (Percent of egg penetrated)	MANCT, SAGE

Statistical Methods.

Two workers had both lead and microwave exposure. These two workers were excluded from all data analyses.

Outcome measures were divided into three categories: primary variables, secondary variables, and descriptors. **Primary variables** represented variables which were expected, based on prior research, to show the most sensitivity to toxic exposures and/or which are most commonly associated with male reproductive potential. The 15 variables identified in this category are provided in Table 3.

Secondary variables are those which were thought to be less sensitive to toxic exposures or which are not as clearly associated with male reproductive potential. The 24 variables in this category are identified in Table 4. All statistical tests of secondary variables were conducted at alpha = 0.05 with no adjustment for multiple comparisons. Changes that were significant at alpha = 0.05 were considered as limited evidence of an adverse effect due to lead or microwave exposure.

For convenience of programming and reporting, covariates were abbreviated to variable names of 8 characters or less. The list of **a priori** covariate abbreviations and associated descriptions are provided in Table 2.

Prior to any data analysis, descriptive statistics were computed on all potential covariates. Covariates which showed a significant change from one study group to another were automatically included in the initial modelling stage for all primary and secondary variables. Any variables which were significantly different between the study participants and the random survey of non-participants were also included in the initial modelling stage.

The **initial model** for each primary and secondary variable was a stepwise regression model. All covariates specified **a priori** for that variable plus any others which showed a significant difference as specified above were included in this model. Categorical covariates were recoded into an appropriate number of indicator variables. Two contrasts (Lead - Control and Microwave - Control) were also included and were forced into the model. Residuals from the stepwise model were examined for normality and equality of variances across all exposure groups. Further, these residuals were plotted against each continuous scale potential covariate (not just the ones selected by the stepwise procedure) to evaluate for non-linearity in the covariate effects. Finally, residuals were plotted against each indicator variable (again not just the ones selected by the stepwise procedure) to examine the assumption of equal variance across the levels of the categorical covariate.

Table 4. List of secondary variables with **a priori** potential covariates.

Secondary variables	Potential covariates
1. DVEL (Average distance velocity)	TEMP, SAGE, CAFFEINE
2. LIN (Average linearity)	same as above
3. ALH (Average lateral head amplitude)	same as above
4. BCF (Average beat cross frequency)	same as above
5. OSM (Semen osmolality)	ABS, SAGE
6. PH (Semen pH)	same as above
7. FERT_IND (Fertility index)	MANCT, SAGE
8. PEN_IND (Penetration index)	same as above
9. TESTRAT (Free/total testosterone)	no confounders
10. MACRO (morphology classification)	HOTBATH, ALCOHOL, SMOKE, TYPNDRWR, FEVER, VENDIS
11. MICRO (morphology classification)	same as above
12. ABSENTH (morphology classification)	same as above
13. TAPERED (morphology classification)	same as above
14. DOUBLEH (morphology classification)	same as above
15. AMORPH (morphology classification)	same as above
16. TAILDEF (morphology classification)	same as above
17. IMMATURE (morphology classification)	same as above
18. OVAL (4 pi area/ perim^2)	ABS, HOTBATH, ALCOHOL, SMOKE, TYPNDRWR, FEVER, VENDIS, RACE
19. VOL (Semen volume in ml)	ABS, SPILLAGE
20. FRC (Fructose concentration)	ABS, SPILLAGE, STDCURVE
21. ZINC (Zinc concentration)	same as above
22. FSH (Follicle Stimulating Hormone)	no confounders
23. PROL (Prolactin)	no confounders
24. TESTTOT (Total testosterone)	no confounders

A **second model** was fit, starting with the model selected by stepwise regression. To this model, the interaction between the two exposure contrasts and any covariate selected by the stepwise model was added. A significant interaction would indicate lack of parallelism for the covariate and the need to make differential adjustments in the covariate for each group. Any interaction significant at alpha = 0.05 was included in the final model.

Descriptors are those variables that are of limited interest to the researcher. There are four variables in this category; they were analyzed by descriptive statistics only. The four descriptors were TEMP (Semen temperature), COLOR (Semen color), TURBID (Semen turbidity), and LIQ (Semen liquefaction). All statistical tests of descriptors were conducted at alpha = 0.05. No covariates were included and no stepwise modelling procedure was implemented for these variables. For descriptor variables, changes that are significant will not be considered as evidence of an effect due to lead or microwave exposure, but may be considered in the planning of future studies.

Transformations were considered **a priori** for certain variables to account for distribution patterns. These variables have been shown to require such transformations in previous studies to satisfy the assumptions of normality. These variables/transformations included log (base 10) transformation for MANCT and TOTCT. For both of these variables, two subjects had a data value of zero. For these subjects, a value of 0.5 (million) was substituted prior to the log transformation to avoid any errors in the program.

One of the continuous covariates, ABS, had values larger than 7 (days). Previous research has shown that a linear relationship between abstinence and certain semen parameters existed only in a range from 1 to 7 days. After approximately 7 days, the effect of additional days of abstinence appears to be negligible. To account for the effect of abstinence leveling off after 7 days, any abstinence values larger than 7 was set equal to 7 prior to any data analyses.

Statistical Comparisons.

The first statistical comparison was applied between the study participants and the non-participant population as a whole.

In order to better characterize the populations studied, a questionnaire was given to random sample of soldiers who did not participate in the study. This survey queried some demographics to see if the participants in this study differed from the population of soldiers at large. The results of this questionnaire are compared to results from participants in this study in Table 5.

First, we see that the racial/ethnic composition of study participants was primarily white, while a random sample of non-participants was predominantly black or hispanic. This disparity in racial/ethnic composition indicates that it may be difficult to generalize the results of this study to the military population as a whole. On the other hand, the consistency of study participants - racial/ethnic composition among the control, lead-exposed, and microwave-exposed subjects - indicated that this factor did not seriously bias the comparisons among these three groups.

Another major potential confounding factor was concern about the ability to conceive. This concern was absent from the non-participants, with none answering yes to the question "Have you ever had trouble fathering a child (a fertility problem)?" and only 12.5% (6/48) answering "don't know or not applicable". The rates were higher for study participants with 13% (4/31) of the controls, 23% (7/30) of the lead-exposed, and 10% (2/20) of the microwave-exposed workers answering yes to the same question. This indicates a selection bias, the tendency of workers who think they may have a problem volunteering more often than the general population. Selection bias makes generalizations to the military population more difficult. Furthermore, the relatively high percentage of lead-exposed workers who believe they have a fertility problem make an unadjusted comparison between that group and the control workers and/or the microwave-exposed workers problematic. On the basis of these results, an indicator variable for fertility concern was included initially in all models.

The age of the subjects, the number of pregnancies fathered, and the number of normal births were reasonably consistent from one group to another, including the random sample of non-participants.

Table 5. Comparison of questionnaire responses between study participants and independent non-participants

	Age (Mean±SD)
C	28.6±6.4
L	27.5±3.8
M	30.6±7.0
N	29.3±4.7

Race/ethnic background

	White	Black	Hispanic	Asian	Total
C	71% (22)	23% (7)	6% (2)	0% (0)	100% (31)
L	53% (16)	23% (7)	23% (7)	0% (0)	100% (30)
M	60% (12)	25% (5)	10% (2)	5% (1)	100% (20)
N	32% (15)	53% (25)	15% (7)	0% (0)	100% (47)

Have you ever had trouble fathering a child (a fertility problem)?

	No	Yes	DK/NA	Total
C	87% (27)	13% (4)	0% (0)	100% (31)
L	73% (22)	23% (7)	3% (1)	100% (30)
M	90% (18)	10% (2)	0% (0)	100% (20)
N	88% (42)	0% (0)	12% (6)	100% (48)

How many pregnancies (normal and miscarriages) have you fathered?

	0	1	2	3+	Total	Mean±SD
C	23% (7)	19% (6)	32% (10)	26% (8)	100% (31)	1.7±1.3
L	17% (5)	27% (8)	30% (9)	27% (8)	100% (30)	2.0±1.8
M	35% (7)	15% (3)	25% (5)	25% (5)	100% (20)	1.6±1.6
N	25% (12)	25% (12)	35% (17)	15% (7)	100% (48)	1.5±1.2

How many of these were normal births?

	0	1	2	3+	Total	Mean±SD
C	26% (8)	35% (11)	29% (9)	10% (3)	100% (31)	1.2±0.9
L	33% (10)	30% (9)	20% (6)	17% (5)	100% (30)	1.4±1.4
M	45% (9)	15% (3)	15% (3)	25% (5)	100% (20)	1.4±1.6
N	29% (14)	29% (14)	35% (17)	6% (3)	100% (48)	1.2±1.0

C = Control
L = Lead
M = Microwave
N = Non-Participant

Prior to analysis of the primary and secondary measures of male reproductive potential, an analysis of potential interactions of covariates among exposure groups was performed. This analysis consisted of a single factor analysis of variance (ANOVA) for continuous covariates (Table 6) and a chi-square test of independence for categorical covariates (Table 7). Potential covariates which show a significant difference under ANOVA or which showed lack of independence under the chi-square test indicate that the covariate would be a possible source of bias if not included in the regression model. The alpha level was set at .05 with no adjustments for multiple comparisons.

Table 6. Descriptive statistics and single factor analysis of variance for continuous variables.

ABS (p-value=.6642)		ALCOHOL (p-value=.3270)	
C	3.2 ± 3.2 (31)	C	4.8 ± 3.6 (31)
L	2.7 ± 1.5 (30)	L	5.5 ± 3.8 (30)
M	2.8 ± 1.5 (20)	M	4.0 ± 3.6 (20)
CAFFEINE (p-value=.3669)		SAGE (p-value=.3528)	
C	4.8 ± 5.0 (31)	C	55 ± 36 (31)
L	6.0 ± 6.0 (30)	L	52 ± 20 (30)
M	7.2 ± 6.9 (20)	M	44 ± 15 (20)
SMOKE (p-value=.1808)			
C	4.3 ± 8.7 (31)		
L	7.1 ± 8.8 (30)		
M	9.3 ± 11.6 (20)		

C = Control
L = Lead
M = Microwave

Table 7. Descriptive statistics and Chi-square test for independence among categorical covariates

SCIENCE (p-value=.666)

	Not Important	Medium Importance	Very Important	Total
C	6% (2)	39% (12)	55% (17)	100% (31)
L	0% (0)	33% (10)	67% (20)	100% (30)
M	5% (1)	40% (8)	55% (11)	100% (20)

MONEY (p-value=.457)

	Not Important	Medium Importance	Very Important	Total
C	19% (6)	55% (17)	26% (8)	100% (31)
L	27% (8)	50% (15)	23% (7)	100% (30)
M	30% (6)	30% (6)	40% (8)	100% (20)

COWORK (p-value=.516)

	Not Important	Medium Importance	Very Important	Total
C	58% (18)	32% (10)	10% (3)	100% (31)
L	77% (23)	13% (4)	10% (3)	100% (30)
M	70% (14)	20% (4)	10% (2)	100% (20)

TEST (p-value=.932)

	Not Important	Medium Importance	Very Important	Total
C	6% (2)	19% (6)	74% (23)	100% (31)
L	3% (1)	23% (7)	73% (22)	100% (30)
M	10% (2)	20% (4)	70% (14)	100% (20)

WHYPROB (p-value=.342)

	Not Important	Medium Importance	Very Important	Total
C	84% (26)	3% (1)	13% (4)	100% (31)
L	63% (19)	10% (3)	27% (8)	100% (30)
M	80% (16)	10% (2)	10% (2)	100% (20)

Table 7 (cont). Descriptive statistics and Chi-square test for independence among categorical covariates

HOTBATH (p-value=.963)

	Never	1-3 times a month	1-3 times a week	4 or more times a week
C	61% (19)	29% (9)	6% (2)	3% (1)
L	60% (18)	27% (8)	10% (3)	3% (1)
M	60% (18)	20% (4)	15% (3)	5% (1)

BCTINFCT (p-value=.460)

	No	Yes	DK/NA	Total
C	97% (30)	0% (0)	3% (1)	100% (31)
L	90% (27)	7% (2)	3% (1)	100% (30)
M	95% (19)	5% (1)	0% (0)	100% (20)

MARIJ (p-value=.533)

	No	Yes	Total
C	97% (30)	3% (1)	100% (31)
L	90% (27)	10% (3)	100% (30)
M	95% (19)	5% (1)	100% (20)

TYPNDRWR (p=.305)

	Boxer shorts	Briefs	No Underwear	Total
C	10% (3)	77% (24)	13% (4)	100% (31)
L	7% (2)	87% (26)	7% (2)	100% (30)
M	0% (0)	100% (20)	0% (0)	100% (20)

VENDIS (p-value=.468)

	No	Yes	Total
C	65% (20)	35% (11)	100% (31)
L	63% (19)	37% (11)	100% (30)
M	80% (16)	20% (4)	100% (20)

Analysis for Specific Covariates.

Tables 8 through 62 present the final results of statistical analysis. Every table includes a listing of the sample size and the unadjusted means for each group. If the statistical analysis uncovered one or more significant covariates, then means adjusted for the covariates are also presented. Finally, the two p-values measure the significance of the change between soldiers with potential lead exposure and control soldiers and the significance of change between soldiers with potential microwave exposure and control soldiers.

For some variables, an additional table is presented. For three of the variables, for example, one or more outliers were detected. While a careful examination of these outliers did not reveal any laboratory or clerical errors, an additional analysis with the outlier(s) removed is reported. This additional analysis is intended only to illustrate how sensitive the statistical analysis is to these outliers.

For other variables, statistical analysis uncovered a significant interaction between the exposure variable and self-perceived fertility concern. In these cases, the potentially lead and/or microwave exposed soldiers who had fertility concerns differed significantly from those without concern. Because of this disparate impact, each exposure group was divided on the basis of fertility concern. Descriptive statistics and p-values were then reported for six rows of the table. The p-values in each table represent comparison of each subgroup to control soldiers with no self-perceived fertility concern.

Sperm Count.

No significant differences were seen in total sperm counts between lead exposed and control subjects (Table 8). The adjusted means of 13 million sperm per milliliter (Table 8) and 14 million sperm per milliliter (Table 9) seen with the potentially exposed microwave group differed significantly from the mean of control subjects. A log transformation was applied to this variable. Two subjects with sperm counts of zero had 0.1 added to their values prior to the log transformation.

Table 8. The comparison of unadjusted and race adjusted log transformed means for number of sperm per milliliter of ejaculate related to exposure potential.

Variable: manct	Sample size	Unadjusted means	Adjusted means	p-value
Control	31	38	35	
Lead exposed	30	25	28	0.50
Microwave exposed	20	15	13	0.0085

Table 9. The comparison of unadjusted and race adjusted log transformed means for numbers of sperm per milliliter of ejaculate related to exposure potential and self-perceived fertility concern.

Variable: manct		Sample size	Unadjusted means	Adjusted means	p-value
Control	No	27	38	36	
	Yes	4	38	34	0.93
Lead exposed	No	23	36	38	0.87
	Yes	7	7.2	9.7	0.014
Microwave exposed	No	18	15	14	0.010
	Yes	2	8.9	12	0.23

The group mean of subjects who were potentially exposed to lead aerosols and identified fertility concerns during questionnaire administration demonstrated statistically decreased manual sperm counts. No difference was demonstrated for lead exposed workers who had no concern about possible infertility.

Potentially exposed microwave subjects who noted no concern about fertility difficulty on interview, were found to have a statistically significant decrement in numbers of sperm per milliliter of ejaculate. The race adjusted mean of 14 million sperm per milliliter for microwave subjects without concern and the mean of 12 million sperm per milliliter for microwave workers with fertility concern both appear notably low. Despite the lower manual count mean (12 million) demonstrated for microwave subjects with fertility concern, statistical significance was not achieved. Failure to demonstrate statistical differences appears related to the small cell size for the group, i.e. 2 subjects. These data reinforce the need for additional study of populations with potential microwave exposures to accommodate larger group population sizes.

The total number of sperm per ejaculate was determined for study participants. There were no statistically significant differences between the race adjusted means when the lead exposed group was compared (Table 10). A statistically significant difference was identified for the subjects with potential microwave exposures. A log transformation was applied to this variable. Two subjects with total sperm counts of zero had 0.1 added to their values prior to the log transformation. Neither subject with zero counts related a history of prior vasectomy.

The adjusted mean total for the combined control subjects of 78 million sperm per ejaculate is representative of other normal populations.[41,42] It should be noted that the total count for the combined microwave exposure group is only 33 million. Although not predictive of male infertility difficulty, a total count of 33 million sperm per ejaculate would be considered a clinically noteworthy finding in a health clinic setting.[42]

Table 10. The comparison of unadjusted and race adjusted log transformed means for total sperm count related to exposure potential.

Variable: totct	Sample size	Unadjusted means	Adjusted means	p-value
Control	31	85	78	
Lead exposed	30	55	63	0.55
Microwave exposed	20	36	33	0.027

Statistical evaluation of the total sperm count per ejaculate based upon both exposure potential and level of self-perceived fertility concern differs from the combined group data (Table 11). Narrowly missed significant differences were demonstrated between lead exposed subjects who noted possible difficulty with fertility ($p=0.067$) and the microwave subjects who identified no fertility concern ($p=0.051$). The adjusted mean for the control group with no fertility concern was not identified as different from microwave exposed subjects with fertility concern. The control group adjusted mean of 74 million appeared different on visual comparison with the adjusted mean of 30 million for fecundity concerned microwave exposed subjects. It is likely that these differences would have been statistically different if a larger study population (cell size) had been evaluated and demonstrated similar adjusted mean values.

Table 11. The comparison of unadjusted and race adjusted log transformed means for total sperm count related to exposure potential and self-perceived fertility concern.

Variable: totct		Sample size	Unadjusted means	Adjusted means	p-value
Control	No	27	79	74	
	Yes	4	140	120	0.48
Lead exposed	No	23	78	81	0.81
	Yes	7	18	26	0.067
Microwave exposed	No	18	38	34	0.051
	Yes	2	22	30	0.34

Sperm Morphology.

Sperm morphology was performed as a subjective microscopic examination of a dry mount slide preparation. Two hundred sperm cells were evaluated for size and shape compared to other cells on the slide preparation. Each sperm cell was placed in a single prioritized counting category for numerical purposes, even if several abnormalities were present in the same cell. While this methodology maintains a total accountability of each sperm dry mount preparation, the method fails to identify the possibility of multiple identified defects in sperm morphology on a single slide preparation.

After adjustment for the frequency of hot baths, no statistically significant differences for the arcsin transformed percentage of sperm with normal morphology were identified when either lead or microwave exposure groups were compared with controls (Table 12). In contrast, significant differences in percentages of normal sperm were demonstrated between the means for both lead (p= 0.019) and microwave (p= 0.032) exposed subjects who identified fertility concern (Table 13) during administration of the study questionnaire. The identified differences are biologically plausible and serve to demonstrate the importance of further study with larger numbers of participating subjects.

Table 12. The comparison of unadjusted and adjusted (for frequency of hot baths) arcsin transformed means for the percentage of normal sperm related to exposure potential.

Variable: normal	Sample size	Unadjusted means	Adjusted means	p-value
Control	30	73	73	
Lead exposed	29	72	72	0.92
Microwave exposed	19	68	69	0.20

Table 13. The comparison of unadjusted and adjusted (for frequency of hot baths and smoking) arcsin transformed means for the percentage of normal sperm related to exposure potential and self-perceived fertility concern.

Variable: normal		Sample size	Unadjusted means	Adjusted means	p-value
Control	No	26	73	73	
	Yes	4	73	72	0.90
Lead exposed	No	23	75	75	0.40
	Yes	6	61	61	0.019
Microwave exposed	No	17	69	70	0.40
	Yes	2	57	56	0.032

No significant differences from the control group were identified between unadjusted and adjusted (for smoking status) arcsin transformed means for the percentage of sperm with large cell size for any of the study groups (Table 14).

Table 14. The comparison of unadjusted and adjusted arcsin transformed means for frequency of large sperm cell size related to exposure potential after adjustment for smoking status.

Variable: macro	Sample size	Unadjusted means	Adjusted means	p-value
Control	30	1.7	1.7	
Lead exposed	29	1.4	1.4	0.33
Microwave exposed	19	1.3	1.3	0.24

Comparison of the unadjusted arcsin transformed means identified no significant differences between exposure groups and the control population with respect to the percentages of small sperm cells (Table 15). Similarly, no differences were identified between arcsin transformed mean frequencies for small sperm size between subjects with and without fertility concerns (Table 16).

Table 15. The comparison of unadjusted arcsin transformed mean frequencies of small sperm cell size related to exposure potential.

Variable: micro	Sample size	Unadjusted means	p-value
Control	30	2.9	
Lead exposed	29	2.4	0.23
Microwave exposed	19	3.1	0.65

Table 16. The comparison of unadjusted arcsin transformed mean frequencies of small sperm cell size related to exposure potential and self-perceived fertility problems.

Variable: micro		Sample size	Unadjusted means	p-value
Control	No	26	2.9	
	Yes	4	2.7	0.88
Lead exposed	No	23	2.1	0.10
	Yes	6	3.3	0.58
Microwave exposed	No	17	3.0	0.80
	Yes	2	3.8	0.51

No statistical differences were identified between the arcsin transformed mean frequencies for absent sperm heads in any of the groups within the study before analysis related to parenting concern (Table 17).

Table 17. The comparison of unadjusted arcsin transformed mean frequencies of sperm with absent head related to exposure potential.

Variable: absenth	Sample size	Unadjusted means	p-value
Control	30	4.7	
Lead exposed	29	3.8	0.20
Microwave exposed	19	4.7	0.98

Analysis of group information with respect to a self-perceived fertility problem (Table 18) resulted in identification of significant differences for both lead, with no concern for parenting difficulty, and potentially microwave exposed subjects with parenting concern. The high number of absent heads in the microwave group (9.4) is statistically different than the expected, and may be associated with a fragile sperm neck section or other defect in spermatogenesis.[42] Although the finding could result from an uncommon artifact during careful slide preparation, the non-random distribution of the finding in the blinded analysis supports the possibility of a biological response to exposure.

Table 18. The comparison of unadjusted arcsin transformed mean frequencies of sperm with absent head related to exposure potential and to a self-perceived parenting concern.

Variable: absenth		Sample size	Unadjusted means	p-value
Control	No	26	4.8	
	Yes	4	3.8	0.45
Lead exposed	No	23	3.3	0.025
	Yes	6	6.3	0.24
Microwave exposed	No	17	4.3	0.47
	Yes	2	9.4	0.045

Double-headed sperm are thought to be formed as a result of incomplete meiosis during spermatogenesis.[42] There were no significant differences between numbers of sperm with double heads of control and potentially exposed group subjects for analyses with (Table 19) or without (Table 20) information related to the two subject outliers. There was one outlier in the self-perceived, potentially lead exposed group and the other was in the self-perceived, potentially microwave exposed group. When analyses were performed with respect to a self-perceived problem, both self-perceived lead (p=0.014) and microwave (p=0.021) exposed group means were significantly different than the control group mean (see Table 21). When both outliers were removed from analysis, there were no significant differences between any of the compared groups (Table 22).

Table 19. The comparison of unadjusted arcsin transformed mean frequencies of sperm with double heads related to exposure potential.

Variable: doubleh	Sample size	Unadjusted means	p-value
Control	30	1.3	
Lead exposed	29	1.9	0.23
Microwave exposed	19	1.4	0.87

Table 20. The comparison of unadjusted arcsin transformed mean frequencies after removal of the two outlier subjects for sperm with double heads related to exposure potential.

Variable: doubleh (w/o outliers)	Sample size	Unadjusted means	p-value
Control	30	1.3	
Lead exposed	28	1.7	0.34
Microwave exposed	18	1.1	0.52

Table 21. The comparison of unadjusted arcsin transformed mean frequencies of sperm with double heads related to exposure potential and to a self-perceived parenting concern.

Variable: doubleh		Sample size	Unadjusted means	p-value
Control	No	26	1.2	
	Yes	4	2.0	0.37
Lead exposed	No	23	1.5	0.48
	Yes	6	3.4	0.014
Microwave exposed	No	17	1.1	0.81
	Yes	2	4.9	0.021

Table 22. The comparison of unadjusted arcsin transformed mean frequencies of sperm with double heads related to exposure potential and to a self-perceived parenting concern after removal of the two study outlier subjects.

Variable: doubleh (w/o outlier)		Sample size	Unadjusted means	p-value
Control	No	26	1.2	
	Yes	4	2.0	0.31
Lead exposed	No	23	1.5	0.42
	Yes	5	2.4	0.12
Microwave exposed	No	17	1.1	0.79
	Yes	1	0.5	0.50

All unadjusted mean values for arcsin transformed frequencies of sperm with tapered heads were lower in this study than customary normal values [42], however, they appeared both real and reproducible within this study. The mean values could represent the presence of a high number of immature sperm in these cases. There were no significant differences for unadjusted or adjusted means, however, between any of the study groups compared (Table 23).

Table 23. The comparison of unadjusted and race adjusted arcsin transformed mean frequencies of sperm with tapered heads related to exposure potential.

Variable: tapered	Sample size	Unadjusted means	Adjusted means	p-value
Control	30	0.41	0.42	
Lead exposed	29	0.53	0.54	0.54
Microwave exposed	19	0.55	0.52	0.62

Sperm which are amorphous have an identifiable head, however, the shape of the head is abnormal. No significant differences were identified between unadjusted means, means adjusted for alcohol consumption (Table 24), or unadjusted or adjusted means with respect to perception of fertility difficulties (Table 25).

Table 24. The comparison of unadjusted and alcohol consumption adjusted arcsin transformed mean frequencies of amorphous sperm related to exposure potential.

Variable: amorph	Sample size	Unadjusted means	Adjusted means	p-value
Control	30	0.81	0.81	
Lead exposed	29	1.0	1.0	0.43
Microwave exposed	19	1.1	1.2	0.23

Table 25. The comparison of unadjusted and alcohol consumption adjusted arcsin transformed mean frequencies of amorphous sperm related to exposure potential and self-perceived fertility concerns.

Variable: amorph		Sample size	Unadjusted means	Adjusted means	p-value
Control	No	26	0.89	0.93	
	Yes	4	0.40	0.23	0.077
Lead exposed	No	23	0.99	0.94	0.96
	Yes	6	1.2	1.2	0.55
Microwave exposed	No	17	1.1	1.2	0.38
	Yes	2	1.2	0.89	0.96

A tail defect is classified as any morphological alteration where the sperm tail has an abnormal appearance. One individual was an extreme outlier. Analyses of the data with and without the outlier revealed no significant differences between any of the study groups. The impact of the single outlier is seen in the difference between p=0.15 and p=0.51 (Tables 26 and 27) with and without the outlier, respectively, in the microwave potential exposure group.

Table 26. The comparison of unadjusted arcsin transformed mean frequencies of subjects for sperm with tail defects related to exposure potential.

Variable: taildef	Sample size	Unadjusted means	p-value
Control	30	4.7	
Lead exposed	29	5.2	0.66
Microwave exposed	19	6.5	0.15

Table 27. The comparison of unadjusted arcsin transformed mean frequencies after removal of the outlier subject for sperm with tail defects related to exposure potential.

Variable: taildef (w/o outlier)	Sample size	Unadjusted means	p-value
Control	30	4.7	
Lead exposed	29	5.2	0.60
Microwave exposed	18	5.4	0.51

Spermatogonia, spermatids, spermatocytes, and sperm containing cytoplasmic droplets are identified as immature sperm. Careful evaluation of the microscopic specimen is necessary to prevent identification of lymphocytes as immature sperm.[42] There were no significant differences between the arcsin transformed means of any of the study groups (Table 28). A p-value of 0.10 was calculated for the self-perceived difficulty/lead exposed group of subjects (Table 29). It is possible that the difference between the mean of 8.4 and the mean of 13 would have been significant if a larger number of individuals had been included in the lead exposed/self-perceived concern group.

Table 28. The comparison of unadjusted and adjusted (for frequency of hot baths and smoking) arcsin transformed means for immature sperm related to exposure potential.

Variable: immature	Sample size	Unadjusted means	Adjusted means	p-value
Control	30	8.1	8.4	
Lead exposed	29	8.5	8.4	0.98
Microwave exposed	19	9.6	9.3	0.60

Table 29. The comparison of unadjusted and adjusted (for frequency of hot baths and smoking) arcsin transformed means for immature sperm related to exposure potential and self-perceived fertility concern.

Variable: immature		Sample size	Unadjusted means	Adjusted means	p-value
Control	No	26	8.2	8.4	
	Yes	4	7.4	8.1	0.90
Lead exposed	No	23	7.4	7.3	0.46
	Yes	6	14.0	13.0	0.10
Microwave exposed	No	17	9.6	9.1	0.70
	Yes	2	10.0	11.0	0.60

Morphometric Analysis.

Comparisons between the control population and individuals with potential lead or microwave exposure was performed with respect to scientifically established parameters related to careful morphometric analysis. Morphometric parameters evaluated in this study were sperm head length, sperm head width, sperm head area, sperm head perimeter, sperm head length/width ratio, and sperm head oval factor.

The comparisons of control population sperm head length related to the potential exposures of lead and microwave exposures demonstrated no statistically significant differences (Table 30). Step wise linear regression identified frequency of hot baths as a potential confounding variable. Comparison of the adjusted means demonstrated no difference with statistical significance.

Table 30. The comparison of sperm head length in micrometers related to exposure potential with adjustment for hot baths as a confounding variable.

Variable: length	Sample size	Unadjusted means	Adjusted means	p-value
Control	29	4.8	4.9	
Lead exposed	29	4.9	4.9	0.40
Microwave exposed	19	5.0	5.0	0.25

The comparisons of sperm head width with control subjects demonstrated no statistically significant differences for either lead or microwave exposure potentials (Table 31). No confounding variables were identified by step wise linear regression analysis.

Table 31. The comparison of sperm head width in micrometers related to exposure potential.

Variable: width	Sample size	Unadjusted means	p-value
Control	29	3.0	
Lead exposed	29	3.1	0.32
Microwave exposed	19	3.1	0.30

No statistically significant differences were identified for the unadjusted or adjusted means for sperm head area related to the potential for lead or microwave exposures. The frequency of hot baths and prior history of sexually transmitted disease were identified by linear regression as potential confounding variables. As a result, an adjusted mean was compared with the conclusion that there were no statistically significant differences (Table 32).

Table 32. The comparison of sperm head area in square micrometers related to exposure potential with and without adjustment for frequency of hot baths and prior history of sexually transmitted disease.

Variable: area	Sample size	Unadjusted means	Adjusted means	p-value
Control	29	10	10	
Lead exposed	29	11	11	0.33
Microwave exposed	19	11	11	0.39

Sperm head perimeter is measured using a computerized analytical program. It is defined by the known linear relationship between the number of cathode ray screen pixels occupied by the sperm head perimeter and cumulatively quantified by the computerized program. No statistically significant differences for sperm head perimeters were identified for either the unadjusted or adjusted means between the control and potentially exposed subject populations (Table 33).

Table 33. The comparison of sperm head perimeter in micrometers related to exposure potential with and without adjustment for frequency of hot baths and type of undergarment worn.

Variable: perim	Sample size	Unadjusted means	Adjusted means	p-value
Control	29	13	13	
Lead exposed	29	13	13	0.17
Microwave exposed	19	13	13	0.11

No statistically significant differences in sperm head ratio were identified between control and potentially exposed study subjects (Table 34). No potential confounding parameters were identified by regression analysis for sperm head ratio.

Table 34. The comparison of sperm head ratio related to exposure potential.

Variable: ratio	Sample size	Unadjusted means	p-value
Control	29	0.63	
Lead exposed	29	0.63	0.75
Microwave exposed	19	0.63	0.84

The oval factor is defined in terms of a mathematic function related to the sperm head area and sperm head perimeter. The formula to derive the oval factor is

$$\text{Oval Factor} = 4\pi\ (\text{AREA})/(\text{PERIMETER})^2.$$

No statistically significant differences were identified for either unadjusted or mean values adjusted for drinking status, i.e. alcohol consumption as defined by questionnaire, related to the sperm head oval factor (Table 35).

Table 35. The comparison of sperm head oval factor related to exposure potential with and without adjustment for drinking status.

Variable: oval	Sample size	Unadjusted means	Adjusted means	p-value
Control	29	0.75	0.75	
Lead exposed	29	0.75	0.74	0.45
Microwave exposed	19	0.74	0.74	0.39

No statistically significant differences were found to be associated with the oval factor between all study groups with and without adjustment. Although a step wise linear regression analysis had identified alcohol consumption as a possible confounder, no statistically significant difference was detectible by comparison with the control population (Table 36). The p-values for individuals with fathering concerns in both the control and potentially lead exposed groups may have been identified as significantly different if a larger study population size had been incorporated.

Table 36. The comparison of sperm head oval factor related to exposure potential and a self-perceived fertility problem with and without adjustment for drinking status.

Variable: oval		Sample size	Unadjusted means	Adjusted means	p-value
Control	No	25	0.75	0.75	
	Yes	4	0.78	0.78	0.092
Lead exposed	No	23	0.75	0.75	0.77
	Yes	6	0.73	0.73	0.12
Microwave exposed	No	17	0.74	0.75	0.88
	Yes	2	0.72	0.72	0.23

Motility Analysis.

Sperm motility is a critical requirement for sperm migration through the cervical mucus and is an essential element for egg penetration.[42] Since sperm motility is measured in percent and since the distribution of recorded values for this parameter does not follow a typical Gaussian distribution pattern, an arcsin transformation was required for analysis. The expected value for percent sperm motility is usually in the range of 60 to 70 percent. The value of 50 percent of sperm with forward progression or 25 percent with rapid linear progression are considered normal by the World Health Organization.[41] Therefore, sperm demonstrating 44 percent motility may be within the lower limits of normal (Table 37). A careful review of the internal laboratory procedure and videotaped recording of the sperm samples for this study confirmed the numeric validity of observations recorded for this parameter. The mean percent motility for potentially lead exposed subjects was not statistically different from the mean for control subjects. The mean sperm motility for the subjects potentially exposed to microwave is on the borderline of significance as a difference from both the control and lead exposed groups. A greater than 10 percent difference in sperm motility is not an expected finding in the subjects with potential microwave exposure, however, a statistically significant difference is not demonstrated. If a larger microwave study population had been evaluated, the effect of potential microwave exposure on sperm motility may have been further clarified.

Table 37. The comparison of an arcsin transformation of the percent of motile sperm related to exposure potential and with and without adjustment for temperature of the semen sample and race.

Variable: pctmot	Sample size	Unadjusted means	Adjusted means	p-value
Control	30	44	43	
Lead exposed	28	46	47	0.51
Microwave exposed	19	33	32	0.059

With respect to the straight-line velocity, a step wise linear regression identified race as a potential confounder. No statistically significant differences were identified between either adjusted or unadjusted means for straight-line velocity between any of the study groups (Table 38).

Table 38. The comparison of straight-line velocity of sperm in micrometers per second related to exposure potential and with and without adjustment for race.

Variable: vsl	Sample size	Unadjusted means	Adjusted means	p-value
Control	30	46	46	
Lead exposed	28	43	44	0.68
Microwave exposed	18	41	41	0.15

Curvilinear velocity represents the more accurate measurement for sperm speed and functional motility.[42] No statistically significant differences were identified between the means for control subjects and the adjusted or unadjusted means for subjects who were potentially exposed to lead or microwave (Table 39).

Table 39. The comparison of curvilinear velocity of sperm in micrometers per second related to exposure potential and with and without adjustment for a self-perceived fertility problem.

Variable: vcl	Sample size	Unadjusted means	Adjusted means	p-value
Control	30	62	61	
Lead exposed	28	62	62	0.76
Microwave exposed	18	60	60	0.65

Linearity (lin) is an expression defined as a ratio of curvilinear velocity and straight line velocity. The specific relationship is defined by the equation:

Linearity = Curvilinear Velocity/Straight Line Velocity X 10

As the calculated ratio approximates 10, sperm swimming is more straight line, or linear, in character. Although the differences between the means for control and exposure groups appear small, there is a borderline statistically significant difference for adjusted means based upon race in both potential exposure groups (Table 40).

Table 40. The comparison of linearity of the of motile sperm related to exposure potential and with and without adjustment for race.

Variable: lin	Sample size	Unadjusted means	Adjusted means	p-value
Control	30	6.9	6.9	
Lead exposed	28	6.4	6.5	0.054
Microwave exposed	18	6.5	6.5	0.065

Comparison of lateral head movements of swimming sperm demonstrated no statistically significant differences between the control group and the exposure group (Table 41).

Table 41. The comparison of the amplitude of lateral head movement of motile sperm related to exposure potential.

Variable: alh	Sample size	Unadjusted means	p-value
Control	30	2.1	
Lead exposed	28	2.3	0.23
Microwave exposed	18	2.2	0.43

Beat cross frequency (bcf) is defined as the rate that the moving sperm head crosses the sperm path. The bcf somewhat reflects the rate and motion of the flagellar tail. Mean determinations for the control and potentially exposed groups were adjusted for race. A significant reduction in bcf was found in both the lead and microwave exposure groups. The difference of beat cross frequency between control and potentially lead exposed subjects with self-perceived fertility problem is statistically significant. For subjects potentially exposed to lead, the mean bcf differences remained significant when subjects were separated into two subgroups based upon analysis related to fertility concern. Similarly, a statistically significant difference was identified between the mean values for the control and potentially exposed microwave groups. The impact of the two individuals with potential microwave exposures and a self-perceived fertility problem is clearly demonstrated by comparison between tables 42 and 43.

Table 42. The comparison of the beat cross frequency of lateral head movement of motile sperm related to exposure potential with and without adjustment for race.

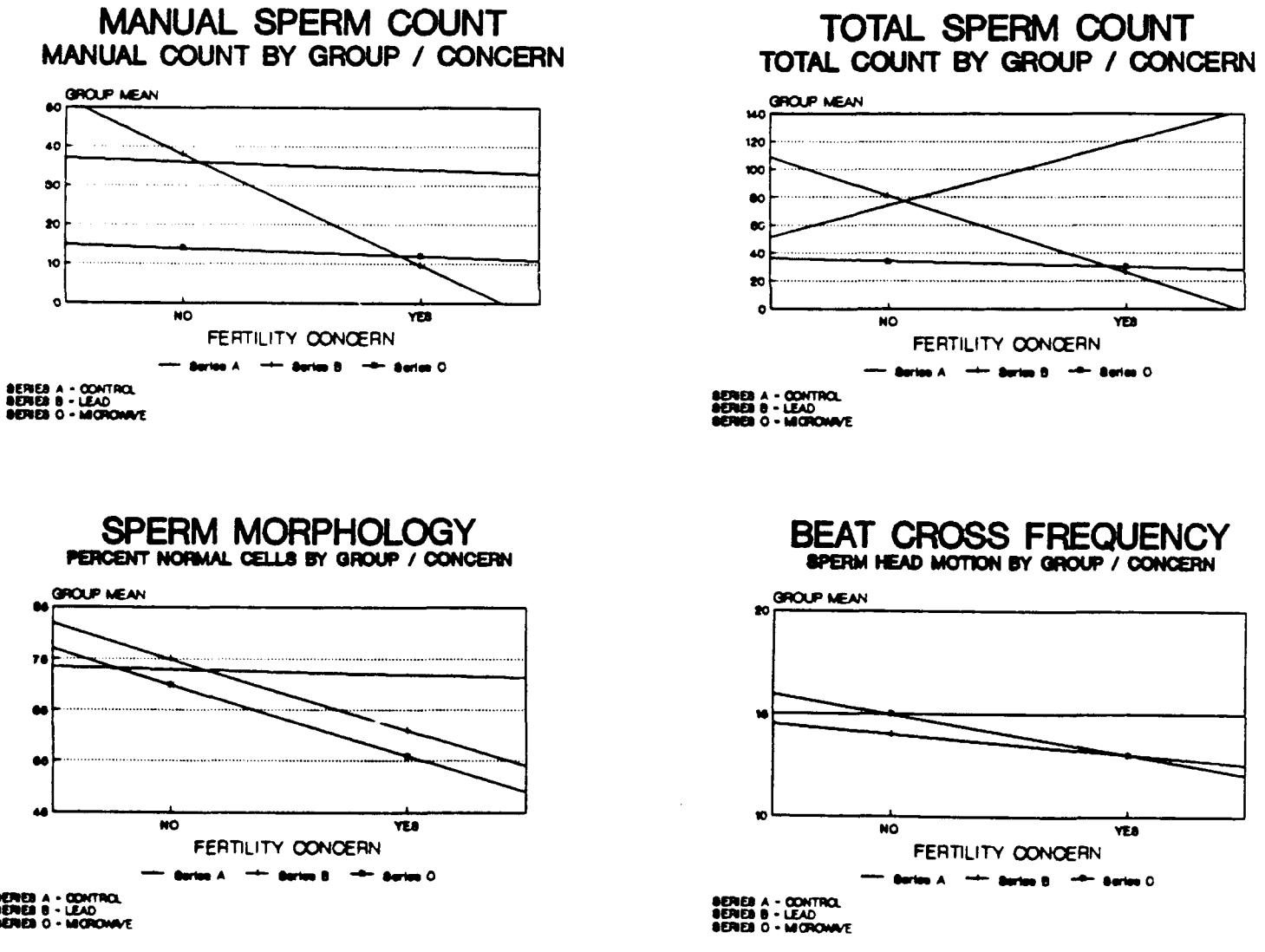
Variable: bcf	Sample size	Unadjusted means	Adjusted means	p-value
Control	30	15	15	
Lead exposed	28	14	14	0.0077
Microwave exposed	18	14	14	0.044

Table 43. The comparison of the beat cross frequency of lateral head movement of motile sperm with consideration of self-perceived reproductive problem and related to exposure potential with and without adjustment for race.

Variable: bcf		Sample size	Unadjusted means	Adjusted means	p-value
Control	No	27	15	15	
	Yes	3	15	15	0.65
Lead exposed	No	23	14	14	0.024
	Yes	5	13	13	0.0017
Microwave exposed	No	16	15	15	0.12
	Yes	2	13	13	0.013

The difference between the subjects with fertility concern remained significant when analyzed as a separate subset. However, the mean for potentially exposed microwave subjects who expressed no concern about fertility difficulty did not differ significantly from the control population. The existence of an unexplained interaction between concern for fertility, potential lead exposure, and potential microwave exposure is suggested by the graphic representation in Figure 1.

Figure 1. Graphic representation for trend with respect to statistically significant differences demonstrated for selected parameters based upon exposure potential and fertility concern.



Viability Analysis.

Analysis of the integrity and function of the sperm cell membrane is evaluated using a staining technique. Sperm cells that do not have an intact membranes fail to exclude entry of dye into the cells. Those cells that become stained represent non-viable cells. No statistical differences were identified between arcsin transformed means of the control group and either potentially exposed group (Table 44). No potential confounders were identified by step wise linear regression.

Table 44. The comparison of an arcsin transformation of the percent of unstained sperm related to exposure potential.

Variable: pctvital	Sample size	Unadjusted means	p-value
Control	30	74	
Lead exposed	28	72	0.57
Microwave exposed	19	69	0.28

The assessment of the functional ability of the membrane to respond to an osmotic challenge has been accepted as an index for evaluation of sperm viability or age. Sperm cells which appear swollen before hypo-osmotic challenge are immature sperm. Those sperm which become swollen in response to a hypo-osmotic challenge are considered viable.[30] Analysis of the data demonstrates that no significant differences were detected between the control population or either group with potential exposure.

Table 45. The comparison of an arcsin transformation of the percent of swollen sperm related to exposure potential and with and without adjustment for race.

Variable: pctswol	Sample size	Unadjusted means	Adjusted means	p-value
Control	30	65	64	
Lead exposed	28	63	64	0.98
Microwave exposed	19	60	59	0.32

Penetration Analysis.

Table 46. The comparison of an arcsin transformation of the percent of zona pellucida free hamster eggs penetrated by sperm related to exposure potential.

Variable: pctpen	Sample size	Unadjusted means	p-value
Control	13	37	
Lead exposed	15	28	0.54
Microwave exposed	9	35	0.93

Penetration of the human egg is an important physiological requirement of functional human spermatozoa. The zona free hamster egg penetration assessment has been developed to afford the ability to assess the functional penetration capability of sperm. Removal of the zona pellucida allows penetration of the egg by more than a single functional sperm cell. Flagellar motion is an important parameter of motility that relates directly to the ability of the sperm to penetrate the egg. The function of sperm is considered acceptable in this test when at least ten percent of eggs are penetrated.[42]

Careful review of the arcsin transformed, unadjusted means of the control group compared to the potentially exposed groups demonstrates no statistically significant differences in penetration rates (Table 46).

In addition, review of the table supports acceptable randomization of study subjects. In order to meet logistical time restraints related to this test, only a subset of sperm could be packed in dry ice and shipped for analysis based upon time of collection prior to 2 PM. The distribution of the ratio of subjects (i.e. 13 of 31 controls, 15 of 30 lead, and 9 of 20 microwave exposed) between the three study groups is consistent with acceptable randomization and blinding within the laboratory phase of the study.

The fertility index is described as a function of the number of successful zona free hamster eggs penetrated with relationship to the number of eggs counted or evaluated. No statistically significant differences were identified between the unadjusted means of control and potentially exposed subjects (Table 47).

Table 47. The comparison of the unadjusted means for the fertility index using zona pellucida free hamster eggs penetrated by sperm related to exposure potential.

Variable: fert ind	Sample size	Unadjusted means	p-value
Control	14	1.1	
Lead exposed	16	0.71	0.26
Microwave exposed	9	0.75	0.39

The penetration index is defined as the number of sperm that have penetrated zona free hamster eggs divided by the number of eggs penetrated.

Although it would have been preferable to perform this analysis for all study participants, it was not possible logistically. As a result, semen samples were randomly selected for hamster egg penetration studies based on a time requirement for shipping and receiving samples in dry ice for analysis.

Table 48. The comparison of the unadjusted means for the penetration index using zona pellucida free hamster eggs with sperm and related to exposure potential.

Variable: pene ind	Sample size	Unadjusted means	p-value
Control	11	2.4	
Lead exposed	14	2.1	0.40
Microwave exposed	8	1.8	0.12

One individual demonstrated a penetration index of 4.11 and, as a result was first included in group comparisons then removed from the subject population as an outlier (Table 49). Analysis of group data including the single outlier demonstrated no statistical differences between group unadjusted mean values.

In contrast, statistical analysis between groups, performed without the outlier, resulted in a highly significant difference in the unadjusted penetration index means between the potential microwave exposure group and the control group (p=0.0085). The penetration index may normally be expected to equal or exceed 4.1 in randomly selected populations.[42]

Table 49. The comparison of the unadjusted means for the penetration index using zona pellucida free hamster eggs with sperm and related to exposure potential after removal of the sole outlier.

Variable: pene_ind (without outlier)	Sample size	Unadjusted means	p-value
Control	11	2.4	
Lead exposed	14	2.1	0.32
Microwave exposed	7	1.4	0.0085

Neuroendocrine Analysis.

Statistical comparison of the log transformation of the unadjusted means of luteinizing hormone demonstrated no statistically significant differences between the control and potentially exposed groups (Table 50).

Table 50. The comparison of a log transformation of luteinizing hormone level related to exposure potential.

Variable: lh	Sample size	Unadjusted means	p-value
Control	30	3.9	
Lead exposed	29	3.5	0.39
Microwave exposed	20	3.7	0.63

Statistical comparisons of the log transformation of the unadjusted means for free testosterone level demonstrated no statistically significant differences between the control and potentially exposed groups (Table 51).

Table 51. The comparison of a log transformation of free testosterone hormone level related to exposure potential.

Variable: testfree	Sample size	Unadjusted means	p-value
Control	30	24	
Lead exposed	29	23	0.75
Microwave exposed	20	24	0.98

In normal males, FSH levels often increase as a stimulus to increase production of sperm.[44] In customary circumstances, the hormone inhibin acts as an inhibitory factor for FSH release.[44] Comparison of the unadjusted mean levels of follicle stimulating hormone revealed no significant differences between study groups (Table 52).

Table 52. The comparison of a log transformation of follicle stimulating hormone (FSH) level related to exposure potential.

Variable: fsh	Sample size	Unadjusted means	p-value
Control	30	3.4	
Lead exposed	29	3.0	0.39
Microwave exposed	20	2.9	0.31

One extreme prolactin hormone level outlier was identified. While there was no historical evidence on questionnaire review to differentiate the cause, stress - such as a difficult phlebotomy - could elevate prolactin levels.[42] The single data point was handled as an extreme value, therefore, and was not used for analysis within study subjects. Both before and after exclusion of the outlier, no significant difference was identified for any study group (Tables 53 and 54).

Table 53. The comparison of a log transformation of prolactin (PROL) level related to exposure potential.

Variable: prol	Sample size	Unadjusted means	p-value
Control	30	5.4	
Lead exposed	29	5.8	0.59
Microwave exposed	20	5.1	0.76

Table 54. The comparison of a log transformation of prolactin (PROL) level related to exposure potential after removal of the outlier from the data set.

Variable: prol (w/o outlier)	Sample size	Unadjusted means	p-value
Control	30	5.4	
Lead exposed	28	5.3	0.90
Microwave exposed	20	5.1	0.72

No statistically significant differences were identified between total testosterone levels for any group (Table 55).

Table 55. The comparison of a log transformation of total testosterone (testtot) level related to exposure potential.

Variable: testtot	Sample size	Unadjusted means	p-value
Control	30	5.3	
Lead exposed	29	5.3	0.95
Microwave exposed	20	5.6	0.53

The testosterone ratio is defined as free testosterone/total testosterone levels. Comparison of the log transformed means between groups demonstrated no significant differences (Table 56).

Table 56. The comparison of a log transformation of means for testosterone ratio (testrat) related to exposure potential.

Variable: testrat	Sample size	Unadjusted means	p-value
Control	30	.0045	
Lead exposed	29	.0044	0.71
Microwave exposed	20	.0043	0.34

Accessory Sex Gland Functional Analysis.

All semen volumes collected and reported for participants of the present study were lower than those customarily seen, often about 3 milliliters volume is minimum.[42] However, volumes in excess of 2.0 ml are considered acceptable by the WHO.[41] An increase in semen volume is usually seen after abstinence or following sexual preparation, e.g. foreplay.[42]

Statistical analysis did not compare volumes in this study with the "normal 3 milliliters", but rather statistical analysis was performed by internal comparison within study subjects. No statistically significant differences were identified between unadjusted or adjusted means for either potentially lead or microwave exposed groups when analyzed as groups (Table 57).

After separation of the study groups into those that perceived a fertility problem, the control subjects who were concerned about fertility were identified as significantly different from other study groups (Table 58). This probably represents a spurious finding, however, since the mean volume of those individuals is consistent with the normally expected volume.

Table 57. The comparison of unadjusted and adjusted means after log transformation for semen volume related to exposure potential after adjustment for race and abstinence time.

Variable: vol	Sample size	Unadjusted means	Adjusted means	p-value
Control	31	2.2	2.2	
Lead exposed	30	2.2	2.2	0.95
Microwave exposed	20	2.5	2.4	0.52

Table 58. The comparison of unadjusted and adjusted means after log transformation for semen volume related to exposure potential with and without adjustment for a self-perceived fertility problem.

Variable: vol		Sample size	Unadjusted means	Adjusted means	p-value
Control	No	27	2.1	2.1	
	Yes	4	3.6	3.5	0.040
Lead exposed	No	23	2.1	2.1	0.82
	Yes	7	2.5	2.7	0.25
Microwave exposed	No	18	2.5	2.5	0.26
	Yes	2	2.5	2.3	0.74

No statistically significant differences were identified between the unadjusted or adjusted osmolarity means of the control group compared to the potential lead or microwave exposure groups (Table 59).

Table 59. The comparison of semen osmolarity related to exposure potential with and without adjustment for age.

Variable: osmol	Sample size	Unadjusted means	Adjusted means	p-value
Control	30	340	340	
Lead exposed	29	340	340	0.89
Microwave exposed	18	330	340	0.85

No statistically significant differences in unadjusted means were identified between semen pH values of the potentially exposed lead or microwave workers in comparison with the control group (Table 60).

Table 60. The comparison of semen pH related to exposure potential.

Variable: ph	Sample size	Unadjusted means	p-value
Control	30	7.9	
Lead exposed	29	7.9	0.97
Microwave exposed	20	7.9	0.48

Semen fructose level is used as a marker of seminal vesicle function.[41] A statistically significant difference for unadjusted group means was identified for semen fructose levels in potentially lead exposed subjects but not for potentially microwave exposed group (Table 61). A careful retrospective review of the laboratory data identified a day to day shift in values of the standard curve developed as the fructose levels were measured. No deficiencies in technique or quality control were identified. As a result of the shift in the "standard curve" adjustment was performed. The difference between the control group mean and the potentially lead exposed group mean remained significant after adjustment.

Table 61. The comparison of unadjusted and adjusted mean semen fructose levels related to exposure potential with and without adjustment for race and standard curve.

Variable: fructose	Sample size	Unadjusted means	Adjusted means	p-value
Control	30	270	280	
Lead exposed	30	370	370	0.027
Microwave exposed	19	300	280	0.91

Table 62. The comparison of unadjusted and adjusted mean semen zinc levels related to exposure potential with and without adjustment for a self-perceived fertility problem.

Variable: zinc	Sample size	Unadjusted means	Adjusted means	p-value
Control	30	12	12	
Lead exposed	30	13	13	0.74
Microwave exposed	19	13	13	0.80

DISCUSSION

This study was performed as a work-site, cross-sectional epidemiologic evaluation of soldiers. It was initiated to characterize the state of reproductive health of artillerymen who may experience significant lead exposures during performance of required military duties. The study was designed to establish an exposure history profile based upon a carefully administered, previously validated questionnaire. Evaluation of blood lead levels, protoporphyrin levels, and hemogram were designed to provide health based clinical confirmation of significant lead exposures. A battery of evaluations to quantify selected hormone levels, characterize semen qualities, and categorize sperm qualities was carefully planned. Sperm characterizations included count, motility, viability, morphology, morphometry, and functional capability.

The study was performed at Fort Hood, Texas, from 20 to 27 July 1990. A total of 28 military occupational specialties were represented from the 24 separate military units that participated in the study. Of the 101 male volunteers who signed consent forms, only 8 subjects withdrew from participation. Ninety three subjects completed at least a portion of the study. While 93 subjects provided partial participation, only 87 participants completed the questionnaires. Since the exposure profile was based upon historical information gleaned from the questionnaire, only those 87 were entered into the initial consideration for analysis. Four subjects were excluded **a priori** from analysis on the basis of historical information. Each excluded individual noted one of the following: (1) frequent welding, (2) frequent hot baths, (3) recent intravenous pyelogram and unilateral cryptorchidism, and (4) questionable historical information.

Of the 83 individuals initially accepted for complete statistical analysis, 2 individuals were not further analyzed because they provided histories of combined potential lead and microwave exposures. There were no adverse clinical reactions or complications reported among study participants.

Pre-selection bias in the study population represented a possible limitation of the present study. Because of the need to recruit as many volunteers as possible during a short time, several different individuals were involved with the request for participation and prospective subject information sessions. On one occasion, statements made by a senior enlisted man resulted in several reactive comments from individuals in attendance at the briefing. In response, almost all members of that ethnic group left the room laughing. The study was soon associated with a street vernacular, negative connotation that possibly resulted in an unknown degree of selective participation of later subjects.

A tremendous amount of questionnaire information was collated and used to categorize the study population. In addition, the questionnaire data were used as an index to assess the self-reported recall responses for potential exposures. The responses were designed to identify lead, microwave, and other physical or chemical interactions that could potentially interfere with sperm production or maturation. Responses of 13 percent of study control participants, 23 percent of lead exposed participants, and 10 percent of microwave subjects indicated a concern for possible fertility problems (Table 5). Those specific individuals who were concerned about reproductive difficulties were not actually confirmed to demonstrate adverse effects in this study. However, the percentage of concerned individuals was consistent with the prevalence of fertility concerns in published reports.[19]

While the non-participant responses to the questionnaire were not representative of the participant population (Table 5), responses did allow limited characterization of the respondents. The responses provided on the self-administered questionnaire characterized the "non-participant" group mean age as comparable to study participants. Non-participants were primarily non-white, in contrast to the participant subjects. While the non-participant survey afforded limited utility for comparison with participants, it did afford responses from a non-randomly selected population of soldiers. None of the non-participants indicated a fertility concern. However, 12 percent of those respondents indicated that they did not know if they were concerned or felt the query was non-applicable. Of the study participants, only one response was seen for the "did not know" or "non-applicable" category; while 10 to 23 percent of the participants indicated some degree of fertility concern. It is unclear whether the participants demonstrated a selection bias for study participation or whether study participation caused a bias by converting the indeterminant response to one of fertility concern.

All questionnaire data were provided by subject recall responses to carefully administered, standardized information solicitations. While the technique of question redundancy afforded an internal mechanism to assist recall, it was not used for a comprehensive, computerized assessment of the reliability of recall. Although it was theoretically possible that an unidentified recall bias could have occurred in a few participating individuals, no adverse impact in study outcome reliability was apparent. The likelihood of significant recall bias that could affect study results is considered remote.

No medical evaluation was performed on study participants. As a result, all health related data were gathered using the extensive study questionnaire based upon the soldier's perception

and recall of his general health and history. It is possible, but unlikely, that significant anatomic abnormalities were overlooked in the history. Subtle causes of oligospermia could easily be overlooked and therefore be under-reported by subjects in the absence of a careful medical examination.

The availability of mobile laboratory equipment has permitted work-site study of male employees to identify potential adverse reproductive functional effects.[18] The authors note that cross-sectional information gained from a single study cannot be reliably used to predict fertility status of individuals or groups of individuals. However, they note that work-site associated impairments among exposed workers may be identified with cross-sectional analysis and may represent an expression of an increased risk of infertility.

Other authors [19] conclude that utilization of semen analysis at the worksite appears to be associated with a number of limitations. Major limitations include obtaining adequate participation of the workforce (workers and controls) and biological variability of semen characteristics. Another variable with a significant potential influence on semen analysis is the length of abstinence time. The difficulty associated with achieving a consistent abstinence time for routine surveillance compared to evaluation in an infertility clinic or one time sample was stressed. It should be noted, however, that it is possible to correct analysis for short abstinence times if the time difference is noted at the time of sample presentation for analysis.[41,42]

A number of advantages of work place, male reproductive studies have been identified. They include: ease of obtaining a large number of sperm cells and early detection of spermatoxic effects prior to infertility. In addition, this type of study affords the ability to identify effects in workers who were physiologically impaired but were not attempting to conceive.

In an effort to promote standardization of research protocols, guidelines have been provided by the Gene-Tox Work Group on Sperm Tests in Animals and Men.[20] Lead was selected as an example of a chemical hazard that has been reported to adversely influence sperm count, motility, morphology and/or Y-bodies. In addition, the Work Group provided a review of the advantages and limitations of sperm evaluations were provided.

Identified advantages of sperm evaluations by the Gene-Tox Work Group [20] included : (1) sperm assessment is related to germinal function and is relevant to check genetic integrity, check fertility, and provide a sensitive indicator of general toxicity; (2) sperm cells may be sampled non-invasively in contrast to oocytes; (3) sperm tests measure chemical effects

in vivo, appear relevant for safety and reproductive assessments, are rapid and relatively inexpensive, and are quantitative; and (4) sperm tests require relatively small samples and are sensitive to small changes.

Identified disadvantages of sperm evaluations by the Gene-Tox Work Group included: (1) relationships have not been defined between small changes in semen quality and adverse reproductive function, mutagenicity, or carcinogenicity; (2) heritability of induced damage is not readily demonstrated in man; (3) cross-sectional sperm analysis in selected groups of individuals may miss transient effects of chemical substances; (4) illnesses, medications, personal habits, or other factors may produce false positive findings; and (5) human samples may be difficult to obtain in certain circumstances.

The potential disadvantages of cross-sectional study of male reproductive function may influence findings. Distinct correlations between small changes in semen quality and adverse reproductive function have not been definitively established. While cross-sectional sperm analysis may miss transient effects of chemical substances and may represent spurious findings as a consequences of illness, medication, or personal habit, it has scientific merit if carefully performed. In addition, early detection of spermatoxic effects may be identified prior to development of permanent health impacts which may alter fecundity potential. Similarly, selected impacts of a toxicant with multi-system pathophysiological insults could resolve, leaving the three month "memory" of an adverse spermatoxic event as the sole residual.

A careful review of the reproductive history, physical examination, and semen analysis represents an acceptable medical evaluation of the male reproductive system.[44,45] The World Health Organization (WHO) has published a manual to assist with laboratory standardization of human semen and semen-cervical mucus analysis.[41] For more sophisticated functional assessment analysis, evaluation of circulating levels of reproductive hormones specialized evaluations of sperm cell function (e.g. hamster egg penetration), careful analysis of multiple components of semen, and computer assisted analysis of sperm characteristics have been used. If considered necessary, the interactions between the semen and cervical mucus can be evaluated using standardized methods in the laboratory.

In the past, most studies of reproductive function have used evaluation of pregnancy outcomes in the study of reproductive toxins.[13] NIOSH scientists report that studies of semen quality may represent a more practical approach to the evaluation of reproductive health because smaller study group sizes are required. NIOSH scientists have estimated that between 16 and 35

subjects should be enrolled to reasonably evaluate possible reproductive effects.[13] While the initial estimate of 25 subjects per group for this study appeared to be within recommended guidelines, analysis of the data has revealed that the study group sizes were sub-optimal.

In the case of this reproductive study, use of mobile laboratory equipment afforded the opportunity to provide an on-site male fecundity study of artillerymen. One clear advantage was the ability to perform almost immediate analyses of a number of semen and sperm related parameters. In addition, the availability of a videotape recorder permitted documentation of sperm movement in a number of microscopic fields for subsequent computerized analysis at a later date. Although this study represents a cross-sectional epidemiologic evaluation, the information gained may potentially provide valuable insight.

The recorded historical and laboratory study data provide insight into the reproductive status for a limited number of artillerymen assigned to the three study groups at a particular point in time. The maximum number of study participants that could be entered into the study was accommodated. However, as a result of study time and resource limitations, the overall ability of subjects to participate was restricted. Although the information gleaned from the study appears important, it is essential that the preliminary nature of the study findings is recognized.

There are a number of excellent publications available that review the spectrum of toxicity associated with lead poisoning. A few of those have been directed toward an overview of exposure potentials, symptoms, signs, and therapeutic alternatives and are useful in general review.[4,5,46,47]

Lead is a ubiquitous metal associated with a rich historical record of beneficial uses and adverse health effects. Current concerns about the toxicity of lead and related impacts on health of human populations are frequently expressed in both scientific and lay literature sources.[1,3,5,6,7,48,49,50,51] Lead associated issues in current scientific and media focus include establishment of safe and acceptable water concentrations for consumption and continued definition of risk/cost benefit analyses. Most recent attention has been directed toward characterization and clarification of the spectrum of potential adverse pharmacological effects and possibility for neurological sequelae in children.

Inorganic lead is primarily introduced into the human body by inhalation or ingestion.[8] In contrast to compounds composed of organic lead derivatives, absorption of inorganic lead across an intact cutaneous barrier is inefficient.[8]

Absorption associated with lead aerosol exposure circumstances similar to those identified for artillery crewmen occurs primarily through inhalation with deposition and retention. Particulates with an aerodynamic In cases where ambient aerosol exposure concentrations were high or when aerosol particulate size favored pulmonary deposition, lead retention was found to be significant. In a recently reported study, inhalation exposures of aerosol particulates was highly correlated with particulate size. Retention in the deep lung was found to be associated with a high degree of systemic exposure through pulmonary absorption thought to approximate 10 times the gastro-intestinal absorption.[52]

Gastro-intestinal ingestion exposures often occur as a secondary event following pulmonary deposition and clearing. Ingestion in this circumstance occurs as a secondary consequence associated with normal broncho-tracheal function and mucocilliary clearance. Pulmonary clearance generates lead containing mucus materials which are subsequently swallowed. Although the gastric acidity favors lead absorption, some excretion of the ingested lead dose occurs in the feces. Absorbed lead is available for deposition in a number of tissues or renal plasma clearance and urinary excretion.[8]

The ILO recognizes an ingestion level of 0.6 mg of lead per day as the borderline level that may result in an increased storage and body burden.[8] They identify the occupational history and laboratory evaluation of hemopoietic changes as primary essentials in early recognition of adverse lead associated effects.

Although medical effects and recommended control measures for lead exposure have been discussed for centuries, recent scientific advances have substantially changed scientific understanding of lead toxicity. It has been recently recognized that toxic effects occur at levels of lead exposure frequently thought to be safe.[43,53] Delayed blood regeneration (secondary to impaired heme biosynthesis), impaired renal tubular function, slowed nerve conduction velocity, impaired central nervous system function, and inhibition of sperm formation have been reported to occur at levels of exposure less than the PEL.[53] As a result, a reduction in the PEL to 20 μg Pb/m^3 of air has been recommended. Similarly, a reduction in the "trigger level" for initiation of employee removal from the workplace has been proposed. Employee removal has been recommended if blood-borne lead levels reach a value of 20 μg Pb/dL of blood. In that circumstance, return of the employee to the work place was recommended when blood lead levels (BLL) had fallen to a level of 10 micrograms per deciliter. Continued overexposure to lead was presented as a preventable national scandal with feasible solutions in the face of ample medical data.

Recent medical literature identifies occupations which are associated with high levels of lead exposure in which inadequate worker awareness, medical surveillance, or biological monitoring are demonstrated.[54,55] Data from the National Occupational Exposure Survey conducted 1981-1983 indicated that approximately 827,000 U.S. workers had potential workplace exposures to lead (exclusive of leaded gasoline). Contaminated work clothing has also been implicated in childhood [54,56] and community exposures.[54]

Current estimates of the automotive repair industry indicate that approximately 435,000 individuals are employed, with about 40,000 directly involved in radiator repair. National Automotive Radiator Service Association studies were performed in seven states from 1979 through 1990. Of the estimated employees, it would appear that 32,000 workers (80%) may have blood lead levels (BLL) in excess of 30 micrograms per deciliter of blood. Forty percent of radiator repair workers are estimated to exceed BLLs of 40 and 21 percent (8400 individuals) are felt to have BLLs above 50 micrograms per deciliter of blood. For workers exposed to lead in California radiator repair shops, only 7.9 percent of employees received routine biological monitoring. Similarly, only 1.4 percent of potentially exposed employees were in positions where environmental monitoring had been performed.[54]

In another report of industries in California, data were interpreted to demonstrate significant lack of compliance with the OSHA Lead Standard.[55] The authors indicate that no effort was made to validate the information provided by self-reporting of employers who provided a 96.7 percent questionnaire response. Only 2.6 percent of facilities engaged in processes using lead were estimated to have performed workplace monitoring. The authors approximate that only 2.6 percent of workers with potential workplace lead exposures participate in routine biological monitoring programs.

Paraoccupational exposures of family members secondary to workplace exposures have been reported.[46,54,56] Workers from radiator repair shops and a worker from a company that made belt buckles have been recently identified who have carried lead into the home from the workplace. In one case, the buckle worker carried sufficient lead into the home to poison his three children. They subsequently required chelation therapy.[56]

Lead exposures above the PEL have been demonstrated in outdoor firing ranges at a police academy associated with cadet training.[57] Collections of breathing zone samples exceeded the eight hour PEL of 50 μg Pb/m^3 of air as a TWA on two separate days. The majority of general area samples, and every breathing zone sample, exceeded the action level of 30 μg Pb/m^3 of air. Although the possibility of inadequate ventilation was noted by the authors, there is the reminder that the location was out of

doors. The main cause of high air lead levels was felt to relate to the use of firing of the conventional, nonjacketed, lead bullets used in cadet training. Substitution of copper jacketed bullets for use at the firing range was recommended.

Lead exposures were characterized for artillerymen and were reported by Bhattacharyya et al.[17] in terms of 24-hour time-weighted averages. Although the customary average is applied over an eight hour workshift, circumstances of the test actually generated an exposure profile over periods of 24 to 96 hours. While the circumstances surrounding military exposures do not represent the customary 8 hour workshift, the ACGIH has endorsed a compensated conversion adjustment [25] to account for the hazards from unusual work exposures. As a result of cumulative toxicity of lead, prolonged exposure periods generate a higher potential for adverse health effect based upon a dosage calculation of concentration of exposure multiplied by the duration of exposure. In addition, prolonged exposures also serve to adversely alter the recovery times (detoxification) necessary between repetitive exposure periods.

Exposure profiles for artillerymen associated with routine firing scenarios have demonstrated that exposure potentials exceed both the PEL and TLV adjusted TWA regardless of the manner of calculation.[17] Maximum excursion limits recommended by the ACGIH[14,58] should not exceed three times the TLV-TWA for more than 30 minutes during the workday, provided that the TLV-TWA is not exceeded for that work shift. Further, the ACGIH recommends that excursions should not exceed five times the TLV-TWA under any circumstances unless adequate personal protective equipment is provided and worn.[58] The ACGIH excursion limit recommendation is based upon the theoretical basis of lognormal distributions of exposure concentrations for otherwise healthy workers.

Military unique lead exposure profiles in soldiers occur as a result of the possibility for high peak exposures separated by various periods of time with limited or no exposure. Exposure potentials have been shown to vary substantially in military unique scenarios depending upon a number of identified circumstances.[17] For example, circumstances that demonstrate differences in possible exposure levels include the following: (1) the type of charge (high zone charges generate higher levels of lead aerosols); (2) type of enclosure (enclosure by crew ballistic shelters increases exposure concentrations); and (3) wind direction (tail winds decrease exposure levels). These types of exposures are difficult to administratively control as a result of the poor ability of a time - weighted average (TWA) to actually reflect peak exposure concentrations. Intermittent, short-term, high-level types of exposure circumstances also cause difficulty with generation of predictions concerning potential health consequences.

The ACGIH has recommended an 8 hour TLV-TWA of 0.15 mg Pb/m^3 of air. Documentation of the scientific and philosophic bases behind the recommendation have been provided.[14,58] The difference between the more restrictive PEL (50 $\mu g/m^3$) and recommended TLV was defended by ACGIH. The possibility that nerve conduction delays could occur as a result of maximal blood lead levels between 50 and 70 μg Pb/dL of blood was presented as the strongest justification, of those advanced by OSHA, for the lower limit.

Biological responses related to the hemoglobin production decrement and altered spermatogenesis were also identified as potential health effects, based upon ACGIH review of the literature.[14] The ACGIH scientists identify possible hematological, neurological, and reproductive effects at blood lead levels of 40-60 micrograms per deciliter. Those levels are reasonably consistent with those recognized and accepted by the ILO.[8] Surprisingly, the ACGIH response to the literature review was that the proposed standard did not recognize hematologic and spermatogenic effects as inconsistent with satisfactory health. The ACGIH position does not address the possible adverse effects related to child-bearing potential associated with altered male reproductive function.

Preplacement, periodic, and termination medical surveillance examinations and biological monitoring for effects of lead exposures have been endorsed by both NIOSH and OSHA. Recommended examinations and monitoring guidelines have been jointly published in the NIOSH/OSHA guideline for inorganic lead exposures.[9] In addition, required examinations and monitoring requirements have been published by OSHA [7] which must be implemented if air-borne lead exposures exceed the action levels. Medical examinations are required as a supplement to employer requirements and are not intended to replace requirements for engineering controls and personal protective equipment. In addition to routine examination and surveillance parameters, the NIOSH/OSHA guideline [9] recommends that the examining physician "...consider...an assessment of fertility, using standardized methods and evaluation criteria."

The blood lead level has served as the principal basis for biological exposure monitoring since its introduction and early application in the 1930s.[46] Changes in blood lead have been previously correlated with lead exposure for artillerymen.[17] The calculated relationship between airborne lead and blood lead was 0.86, approximately 1 $\mu g/dL$ change in blood lead for each μg Pb/m^3 of air. The decline in blood lead levels following exposure was felt to be slower than predicted in previous medical literature. It is important to note the observation that blood lead levels identified in soldiers were representative of levels seen in the general population.[17] It is also important to note

that small, but statistically significant, changes in hematocrit and FEP appeared to be associated with those blood lead changes. The small, transient changes are not expected to demonstrate a clinically relevant finding at these levels of hematological alteration. These findings do indicate, however, that intermittent exposures to high levels of airborne lead may cause changes in human biological function which persist for some time following exposure. The formation of red cell elements demonstrated a transient lead-related impact at lower than anticipated blood lead levels for at least 6 weeks after the exposure was terminated.

In addition to changes in the hemogram, transient changes in protoporphyrin (PP) levels were found to be associated with artillery firing.[17] In a prospective study performed in an industrial setting, lead exposure associated PP level changes were evaluated.[11] The study was designed to investigate the relationship between blood lead and PP levels in workers associated with a smelting operation. Repeated blood analyses were performed and reported for individuals who returned to the smelting operation. Workers were shown to have an increasing blood lead concentration associated with their occupational exposure prior to the elevation of PP levels in peripheral blood. An initial fall in PP levels, followed by the subsequent rise with exposure time, was felt to be consistent with delays of 0 - 21 days reported in scientific literature. As the study progressed, blood lead levels continued to rise while PP levels increased for 25 weeks before they were found to fall slightly. The authors speculated that the inconsistency may have been related to a large variability of PP levels relative to blood lead values.

Important differences between the military unique exposure scenario and the continuous workplace exposure in a smelting operation are readily apparent. These include both the transient "peak and valley" exposure profile for military operators and the contrasting sustained TWA exposure profile for smelter workers. It is probable that the differences observed between transient ZPP responses associated with firing missions [17] and the more prolonged responses in smelting operations [11] are related to the exposure profiles.

There were no actual measurements of either lead or microwave exposure during the course of this study. Inferences concerning microwave exposures may be drawn from the system specifications for microwave system antennae that soldiers in their occupational specialty would be expected to routinely use. Technical Bulletin 430133 provides a comprehensive list of military equipment and normally associated potential microwave exposure characteristics.

Exposures of artillerymen to lead aerosols should be roughly comparable with lead exposure profile determinations from previously performed studies with howitzers. Composition of combustion products, based upon blast or breech data measured during firing of M109 and 8 inch howitzers should approximate lead exposures of artillery crewmen. While the determinations of previous exposures do not afford direct quantification for the actual exposures of the current study participants, they do enable reasonable approximations of exposure in the current population. Factors such as wind speed, wind direction, rapidity of fire, distance from muzzle or breech, type of enclosure (e.g. crew ballistic shelter), type of charge, and work rate have been identified as primary determinants of potential exposure.

Although the questionnaire was extensive and had been previously validated in a successful NIOSH study, inadequate information was recorded to characterize the unique exposure potential differences for the participants in this study. The failure of the questionnaire to characterize the exposure profile was the result of inadequate questions related to specific exposure scenario. For example, questions designed to more optimally characterize exposures should include types of charges fired routinely, normal durations of firing cycles, and exposure frequency. Questions concerning experiences over the last 90 days in a validated, standardized, reproducible manner should have been asked.

In the case of microwave exposure potentials, pre-selection of a group of individuals with military unique microwave exposure profiles was not anticipated. As a result, questionnaires were not prepared in a manner that offered focused, applicable, military potential microwave exposure survey queries. The selection of soldiers performing military intelligence missions to serve as control subjects for bone deposition of lead and nerve conduction evaluation was scientifically reasonable. However, the potential for microwave exposure in the entire "control" group population severely compromised the assignment as "study controls" in the reproductive portion of the study. Because of the late identification of the window of opportunity to perform the reproductive study associated with lead, insufficient time was available to coordinate with Argonne Laboratory scientists. As a consequence, the potential microwave associated group of soldiers were unknowingly included as a control population.

Under the pre-study circumstances, it was impossible to coordinate protocol reviews, identify the frequency rate of microwave exposures, and change the pre-selected military occupational specialty control group identifier, i.e. military intelligence. The potential for microwave exposures was identified by the interviewer during questionnaire administration. Prior to initiation of the study, however, the

possible proximity of military unique non-ionizing radiation sources to soldiers was recognized. As a result, the potential for microwave exposure was included as a possible study dependent covariate. After recognition of the potential impacts of the microwave exposure study population, it was considered unethical to abort the study and prudent to solicit additional soldier control subjects. Similarly, it was considered neither prudent nor ethical to modify the questionnaire in the middle of the study to further characterize a subgroup of exposures within study participants. An additional concern with absolute characterization of microwave exposure circumstances was related to the possibility that some types of security sensitive microwave sources could exist. As a result of questionnaire omissions, the specific delineation and assessment of exposure potentials was suboptimally characterized.

In the present study of reproductive function, no statistically significant relationships were identified between historical lead or microwave exposures and hematologic parameters measured. Specifically, there were no statistically significant changes in blood lead levels or hematologic parameters including complete blood count, FEP, and ZPP associated with potential lead or microwave exposure among study participants.

Although adverse reproductive findings have been associated with lead,[5,6,12,43,59] the relationships between exposure levels, protoporphyrin levels, and other effects remain poorly characterized. Based upon limited data, OSHA [7] required informing and managing individuals of reproductive age at lower levels of blood lead than is required in individuals without reproductive potential. The specific focus of this study was directed toward an assessment of male reproductive system function to quantify changes in semen characteristics as a result of lead exposures.

Evidence showing adverse effects of lead on human reproductive function has been accumulating since before the turn of the century.[6] For example, severely lead-poisoned pregnant women were reported to have an increased rate of spontaneous abortions as early as 1860.[6] There has been increasing concern, over the past two decades, for the potential male and female reproductive complications associated with occupational, paraoccupational, and environmental exposures to lead.[46,60] Adverse effects such as permanent sterility, reversible decreases in fertility, and spontaneous abortions resulting from zygotes conceived by genetically altered spermatozoa have been identified to have a causal relationship with lead exposures.

Adverse effects of lead poisoning in the female include decreased fertility, menstrual abnormality, miscarriage, stillbirths, and increased neonatal mortality.[61] Both

preimplantation and postimplantation reproductive losses have been associated with lead acetate exposures in male rats.[62] In a study designed to maintain the BLL at approximately 70 micrograms per deciliter of blood in female rhesus monkeys, the effects of lead on ovulation and the luteal cycle were evaluated.[61] Female monkeys receiving lead exhibited longer menstrual cycles, variable menstrual cycles, shorter menstrual flow, and suppressed luteal function. There were no significant difference in anovulatory cycles. In a similar study performed earlier, abnormalities in the menstrual cycle were reported to persist after lead exposures were discontinued.[63]

Several scientific reports have been primarily directed toward the review of lead associated male reproductive effects.[43,59,60,64] In a recent meeting of the Society of Toxicology, the study of potential male reproductive effects of lead were identified as "an enormously neglected area in toxicology."[65]

Evaluation of the male partner of an infertile couple has been recommended based upon algorithms that include parameters of semen and hormonal characteristics.[66,67] Although the study of artillerymen performed on the basis of potential lead exposures did not incorporate the precise algorithms recommended, the study protocol was similar. Several additional blood, and a number of additional semen/sperm, parameters were evaluated in this collaborative effort with NIOSH. The UCLA conference proceedings provide an excellent overview of male infertility issues,[67] but are not specifically directed toward chemical toxicant effect. The use of the evaluation of spermatogenesis as a sensitive indicator of potential adverse effects of chemical substances on reproductive function has been recommended.[12,68,69]

The prevalence of individual couples in the general population with concerns related to infertility should be considered in evaluation of the findings of this report. In one worksite study, authors found that fifteen percent of the males in the study population demonstrated oligospermia (sperm counts below 20 X 10^6). While those results were similar to those reported from vasectomy clinic findings, they were higher than findings in other combined occupational studies.[19]

A recent review by the Agency for Toxic Substances and Disease Registry (ATSDR) provides an integrated summary of the prenatal and postnatal effects of lead on children.[70] The review identifies erythropoietic, neural, renal/endocrine, and hepatic effects of lead on children. Adverse reproductive effects are not identified, probably because most data for children are directed toward effects on childhood prior to puberty.

It is possible that adverse effects could be seen in the reproductive maturation of prepubertal males if the Wistar rat model is predictive. While the prepubertal reproductive effects may be of limited value, the report serves to identify the increasing awareness of the toxicity of lead in childhood. The childhood sensitivity to lead is important to identify, however, because of the ability for employees to cause household para-occupational exposures as a result of wearing heavily contaminated clothing into the home.

Studies demonstrated reductions of sperm count and testosterone level in 52 day old male Wistar rats associated with administration of dietary lead acetate.[71] The study was designed to increase lead acetate exposures in the male rat to provide additional data and confirmation of a similar study reported in 1985.[72] In the more recent study, lead exposed rats were given ad libitum access to a solution of 0.6 percent lead acetate rather than the deionized, distilled water provided for drinking in the control animals. Administration of lead was targeted to coincide with the onset of puberty in the young rat. After seven days of administration, there were no differences between the experimental and control groups. However, statistically significant differences were identified at subsequent 14, 30, and 60 day observation times.

Although the sperm counts and testosterone levels were significantly diminished in lead-treated, young, male Wistar rats compared to the control group, the absolute deficiencies remained stable rather than progressively decreasing with time.[71] The author concluded that the recent study confirmed the earlier findings. Based upon the study results, she further concluded that the target organ, i.e. site of action, for lead acetate appeared to be at the level of the hypothalamus in the maturing rat.

Hormones associated with male reproductive function include follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, and testosterone. Accepted norms [42] for these hormones are provided in Table 63. A number of factors could result in finding concentrations outside the accepted range. These include normal physiological fluctuations, stress reactions (most notably prolactin), medical conditions, and chemical exposures. Changes in human male testosterone and prolactin levels have been associated with lead toxicity.[43,73]

Table 63. Accepted serum concentrations of selected hormones in males [42]

HORMONE	ACCEPTED RANGE
Follicle Stimulating Hormone	1.0 to 8.4 mIU/ml*
Luteinizing Hormone	1.0 to 10.5 mIU/ml
Prolactin	2.3 to 11.5 ng/ml
Testosterone	11.0 to 26.0 pg/ml

* mIU = milli International Unit

The reproductive - endocrine axis or spermatogenic mechanism may represent one of the most sensitive target organs of chemical toxins that demonstrate the potential to cause adverse reproductive effects. The possibility for increased susceptibility of the male reproductive system to the toxic effects of lead has been noted.[12] For example, lead may exert toxic male-reproductive effects at low doses, similar to dibromochloropropane (DBCP). The possibility that DBCP may cause severe spermatogenic effects at lower doses than those required to cause other subclinical or overt signs of intoxication has been reported.[68,69] In the absence of other effects, major reported effects of DBCP exposure include azoospermia, oligospermia, and elevated levels of FSH and LH.[21]

In a study of 23 lead smelter workers with a history of lead exposure, the free testosterone index (testosterone/serum binding globulin) was considered to be the best marker of testicular dysfunction.[73] The authors suggest that the first adverse reproductive effect on the hypothalamic-pituitary-testicular axis from chronic lead exposure is direct toxicity to the testicles affecting testosterone synthesis. They note, however, that another toxic action of lead occurs at the hypothalamic or pituitary level. In another study, Sertoli cells were exposed to various concentrations of lead acetate *in vitro*.[74] Metabolic and microscopic abnormalities were associated with lead exposures and were thought to play a role by altering spermatogenesis at the cellular level.

No statistically significant relationships were identified in the present study between historical lead or microwave exposure profiles and the levels of neuroendocrine hormones measured. Similarly, there were no identified differences between the exposure groups and the calculated testosterone ratio (free testosterone/ total testosterone levels).

The process of spermatogenesis in the human male involves a cycle of production and genetic material reduction/distribution, followed by maturation of spermatozoa over a period of approximately 90 days.[44,60] The production cycle, which generates millions of sperm per day, is characterized by rapid cellular division and early sperm development within the testis (approximately 70 days). Completion of the maturation phase occurs over a period of 7 to 21 days within the epididymis prior to ejaculation. A complex functional relationship between production, maturation, secretion, and intact neurological mechanisms is imperative for the integration of interactions necessary for successful erection and ejaculation. Semen secretion components are contributed to the ejaculate by secondary sex glands such as the prostate gland. Accepted variation and standardization of analytical procedures related to sperm and semen have been published.[41]

A firearms instructor at a police academy presented for evaluation of infertility.[75] Although he had fathered a child several years previously, attempts to conceive another child had been unsuccessful. He related complaints of dizziness, headache, irritability and insomnia. After careful evaluation, the patient was found to have an elevated BLL of 80 μg Pb/dL of blood which was responsive to chelation therapy. His initial semen evaluation demonstrated low sperm concentration, low sperm count, poor motility, and morphological abnormalities. A continued improvement in sperm related parameters was demonstrated over a 3 year period, after chelation therapy . Approximately 6 months after chelation, the BLL had declined to about 30 μg/dL and sperm count had improved to 41 million per milliliter. The patient and wife enjoyed a successful conception about one year after chelation. Two and one half years after chelation his reproductive evaluation revealed a total sperm count of 110 million with good motility and 61 percent normal morphology.

It is possible that transient spermatogenic effects from exposures to chemical toxicants could be identified in a cross-sectional study. The spermatogenic development cycle could serve as a biological memory to monitor or register a single adverse toxic experience or series of insults over the 90 day division, maturation, and release period.

Alterations in semen characteristics have been previously reported for a group of soldiers who had a history of Vietnam service.[76] The Vietnam Experience Study was performed as a congressionally mandated health study of Vietnam veterans. The study, performed by the Centers for Disease Control in 1985 and 1986, compared semen analyses between 324 Vietnam veterans and 247 U.S. Army veterans who had no experience in Southeast Asia. Although no specific relationships were identified with combat experiences or agent orange exposure potentials, those veterans

with Vietnam experience were found to demonstrate significant differences in semen parameters. Specific differences included lower mean sperm concentrations, a higher rate of sperm concentrations less than 20 million per milliliter, and lower mean proportions of morphologically normal sperm.

The reproductive ability of males employed in a storage battery plant was evaluated by study of 150 employees.[12] Analysis of the data was performed based upon the blood lead levels. Four potential categories of exposure were identified based upon complaints in association with results of the clinical and toxicology tests. The spectrum of BLL values were then identified in the three groups with highest exposure groups (mean BLL 74.5, 52.8, and 41) and a "physiologic absorption in a polluted environment" group with a mean of 23. Review of the standard deviations demonstrate that overlap occurred between adjacent "exposure" groups. Notwithstanding, clinical complaints and significant differences in sperm parameters were identified between the groups. A control group of 50 individuals without a history of occupational exposures to toxic materials was selected for comparison with the exposure groups. Of the lead exposed employees, 75 percent were considered hypofertile with 50 percent considered infertile. Findings of asthenospermia, hypospermia, oligospermia, and teratospermia were identified and were correlated with the potential for increasing lead exposures. No differences were identified for 17-ketosteroid eliminations between the exposed and control populations.

Oligospermia is currently defined as a sperm count below 20 million sperm per milliliter of ejaculate.[19,64,67] In an attempt to define clinically meaningful sperm counts, authors of one early study compared sperm counts obtained in ejaculates of 4122 pre-vasectomy patients with those of 1000 infertile males.[77] They concluded that if other parameters were normal, sperm counts above 10 million per milliliter and 25 million per ejaculate could not be considered as a major cause of a couple's infertility. A number of possible reasons have been offered as potential causes of aspermia or oligospermia.[20,42] They include normal periodic cyclic variation, frequent ejaculation, incompletely captured ejaculate, withdrawal collection technique, anatomic abnormalities of reproductive structures, and vasectomy. Additional potential causes are related to direct adverse effects upon testicular function by high ambient scrotal/testicular temperature, infection, medication, and exposure to chemical substances.

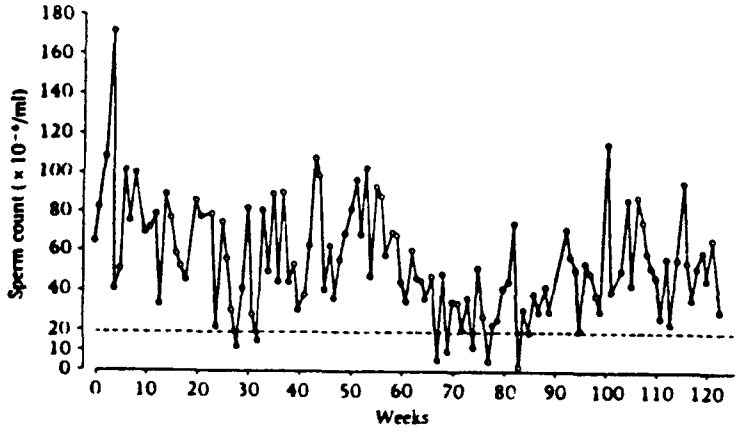
The effects of lowered sperm concentrations and fewer numbers of normal cells upon reproductive function are not clearly established. There is general agreement, however, that reductions in sperm quantity and quality appear to be associated with reduced fertility.[76,78] In one study, the pregnancy rate of

couples where sperm concentrations were performed, was 37.5 percent in the group with motile sperm counts below 5 million per milliliter of ejaculate.[78] The fertility histories between Vietnam veterans and the control group revealed no differences in fathering success, despite the differences in sperm characteristics. Although some individuals suspect that morphologically abnormal sperm may result in an increased rate of spontaneous abortion, no difference was identified between the study groups. The differences between the study groups could not be related to potential agent orange exposures as related by historical recall, nor could any other causal association be identified.

Time dependent longitudinal changes in selected semen characteristics have been recently reported for a group of workers followed for a period of 1 year.[65] In another report, the serial changes of sperm counts were demonstrated in one individual over a period of 120 weeks.[41] Although the mean values for the selected parameters remained within a defined range, a cyclic periodicity was identified. Therefore, the possibility of cyclic change should be considered in individual subject analysis. There is a less dramatic change of group means over time[65], however, because the averaged data obscure the random cyclic phenomena of individual study subjects.

An example of cyclic variability of sperm counts is reproduced from unpublished data from C.A. Paulsen.[41,42] Biweekly seminal fluid sperm concentrations were obtained from a single individual over a period of 120 weeks. Findings are graphically reproduced in Figure 2.

Figure 2. Biweekly seminal fluid sperm concentrations from one individual over a period of 120 weeks



In Figure 2, the dotted line represents a sperm count of 20 million sperm per milliliter of ejaculate. The patient used no medications and reported no febrile illnesses during the investigation period. Based upon the longitudinal experience demonstrated in the figure, notable variability was demonstrated between the highest and lowest recorded values. The demonstrated variability provides scientific evidence that a single sperm count analysis is an inadequate representation of diminished reproductive potential.

In the present study, those individuals who provided a history of potential lead exposure demonstrated no significant alterations in sperm counts as a group. When analyzed as a race-adjusted sub-group, however, those individuals with possible lead exposure who had concern for infertility had significantly lower total and manual sperm counts.

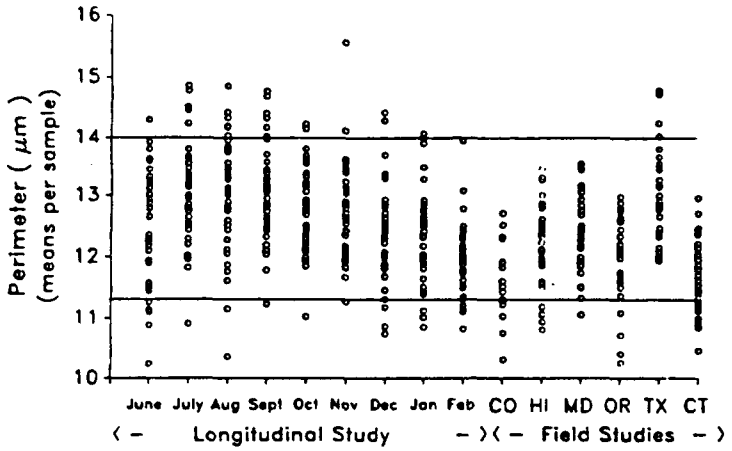
Individuals who provided a history of possible microwave exposure demonstrated significantly fewer sperm than controls in both manual and total counts. The significant difference remained following adjustment for race in the unconcerned infertility group. Although the difference was not considered significant by statistical analysis for the fertility concerned group, the statistical finding is severely restricted by the small group size (cell size = 2). The mean manual count of 12 million sperm per milliliter would clearly be regarded as low in the setting of medical evaluation of male fertility.

During the past 30 years, numerous schemes have been published to formalize the procedures for assessment of normal and abnormal sperm morphologies. Morphological descriptions of sperm size and shape are represented by a continuum of visual microscopic observations rather than distinct, clearly defined, characteristic differences. It has become imperative to establish a validated and reproducible system for scientific and morphological classification of sperm within and especially among laboratories to afford comparability.[79,80,81,82] With recent advances of computerized image analyses, several methods for more precise quantification and measurement of sperm morphometric parameters have been introduced.[45,83,84,85,86,87,88] These systems for morphometric analysis provide objective and reproducible assessments of individual sperm head size and shape. Comparisons of measurements between different analytical systems should be avoided. Sperm morphometry is now often routinely used as part of the assessment of reproductive hazards to the male worker.[17]

In a recent report,[65] sperm morphometric analysis - based upon the longitudinal time course in a single population and comparisons between several normally distributed populations - has been provided. A number of morphometric measurements have been shown to demonstrate a degree of cyclic variability.

A graphic portrayal of data for measured sperm head perimeter demonstrating longitudinal periodicity of a population of workers is provided in Figure 3.

Figure 3. Tolerance intervals for sperm head perimeter measured in a selected population of individuals during a nine month period compared with cross-sectional data from six separate populations [65]



Soldiers from the control group population of the present study were incorporated as the group identified by the acronym "TX" in Figure 3. Soldiers of the control group from this study were found to have large sperm perimeter sizes, similar by comparison with the July - August time frame of the longitudinal study population group.

In the present study, individuals who provided a history of concern about fertility demonstrated significantly fewer normal appearing sperm after adjustment for numbers of hot baths and smoking in both lead and microwave exposed groups. The finding of increased frequency of "absent head" was statistically significant for individuals with lead exposure who related no fertility concern and microwave exposed with fertility concern.

Relatively limited information is available for changes in male reproductive function at the worksite with respect to time. The findings identified among study participants may actually represent an entirely normal form of periodic or cyclic change. A recent study demonstrated that longitudinal changes were demonstrated for selected semen parameters in a group of workers followed for 1 year.[65]

The number of motile sperm is used as a parameter for the evaluation of male reproductive function. Sperm counts have

limited utility unless an adequate description of the motility status of the cells has been given.[64] Diminished motility may be caused by external or internal factors. External factors include use of lubricant in collection, collection by withdrawal, transfer of ejaculate between containers, temperature shock by heat or cold, and ejaculate age. Internal factors include varicocele, infection of secondary sex glands, medications, and chemical exposures.[42] Surgical intervention to correct varicocele has been reported to result in subsequent improvement in motility of sperm.[64] Additional factors that may adversely affect reproductive function include trauma, cryptorchidism, alcohol use, marijuana use, autoimmunity, and high fever.[60]

Workplace exposures to chemicals such as DBCP and ethylene dibromide (EDB) have been reported to exhibit adverse male reproductive effects. In a study of males with occupational exposures to EDB, decreases in semen volume and sperm velocity were identified with early exposures. Increasing durations of exposure to EDB were shown to result in decreases in sperm motility and viability.[89] The authors speculate that the short term exposures may decrease sperm velocity while more chronic exposures will result in sperm immobility and sperm cell death.

Differences in linearity of sperm movements were near levels of significance for unadjusted group means of either lead ($p = 0.054$) or microwave ($p = 0.065$) potential exposures. Beat cross frequency (bcf) was significantly impaired in both unadjusted and race adjusted group means for both lead and microwave groups. Adjusted means for both fertility concerned and unconcerned lead exposed groups were significantly lower than control means. In addition, the group mean of the microwave exposed individuals who expressed fertility concern was significantly lower than control values.

The accumulation of lead within the epididymis could alter its function with the potential to affect maturation of the spermatozoa.[90] The hypothesis was tested by comparing nucleic acid stability in spermatozoa of 9 week old male rats provided ad libitum oral consumption of lead chloride treated drinking water and control animals provided deionized water. Cytochemical staining of the spermatozoa demonstrated condensation and stabilization of the chromatin. The authors suggest that the stabilized chromatin is formed as a result of lead toxicity as the sperm mature while passing through the epididymis. They hypothesize that stabilized chromatin may delay decondensation and result in impaired fertility.

Other authors identify the cauda epididymis of the male rat as the suspected target organ affected by lead exposure.[91] The authors identify the precision of blood lead values, but note that lead levels in some tissues is probably higher than the

blood level. They could identify no change in the number of each type of testicular germinal cell (e.g. spermatogonia, mature spermatozoa) associated with lead dose. Lead exposures were associated with a reduction of spermatogonia and an increase in the number of abnormal sperm cells within the cauda epididymis. Based upon relatively small changes in testicular nucleic acids and protein, the epididymis and testicular germ cells were believed more resistant to lead toxicity.

In the present study, accessory sex gland function was assessed by laboratory analysis of semen zinc and fructose levels. The mean level of semen fructose was significantly higher in the group of individuals who provided a history of lead exposure.

It has been shown that animals exposed to some mutagens have an increase in single stranded DNA [92,93,94] indicating an increase in genetic damage. A recent review of the associations between lead exposure and human teratogenesis and mutagenesis has been published.[95] The fertility rate of bulls is correlated with the percentage of double stranded DNA.[96] A recent report indicates that the DNA stability assay is highly repeatable between ejaculates from the same man.[97] While this procedure has been developed in only a few laboratories, the sperm can be frozen on dry ice and shipped to the laboratory for analysis without affecting the results.

In a heterozygous test, the ability of the human sperm to penetrate the zona pellucida-free hamster egg has been used to evaluate fertility.[98] The results of penetration tests demonstrate a correlation with sperm count and morphology. The authors found that the sperm penetration assay did not yield additional information concerning the cause of infertility in unselected males. The authors conclude that the test impracticality limits the usefulness of the test as an indicator of male infertility.

In this study, the penetration index was found be significantly diminished in the group of individuals with potential microwave exposures, after removal of a single outlier from the analysis. Inclusion of the outlier (penetration index = 4.11) resulted in increasing the group mean from 1.4 to 1.8, enough to counteract the finding of a significant difference. The finding of a lower penetration index has not been definitively correlated with clinically impaired fertility and is of uncertain clinical significance.

It is important to recall that a single study cannot be used to reliably predict fertility status of either the individual subjects or other groups of soldiers performing similar military occupations. Assessments of semen and sperm characteristics have

been scientifically and clinically accepted to adequately represent study of germinal function. Although they have been associated with the evaluation of fertility, a single semen sample cannot reflect the cyclic periodicity [13,41] and should not be used as a definitive measure of male reproductive function. A minimum of two tests demonstrating sperm counts less than twenty million or unusual variability of sperm morphology or viability should be documented for each individual before any statement concerning fecundity is made for that individual. However, it is possible that the differences demonstrated in individuals identified in a cross-sectional study with low sperm counts or morphological variabilities of sperm could have reproductive difficulty.

Sperm assessment may provide a sensitive indicator of general genetic toxicity related to physical or chemical toxicant exposure. As a result, findings identified in the individual participant or group mean semen and sperm analyses may represent important clinical information, but should be interpreted with care. No statement related to individual fertility potential can be made based upon combined group mean data. Similarly, the limitations in universally applicable conclusions based upon a single determination of combined mean group data are apparent. However, findings identified in cross-sectional studies may be extremely useful if used to augment and focus planning for follow-on, prospective, carefully administered studies.

In the present study, no specific causal correlations between actual lead or microwave exposures and adverse reproductive effects have been demonstrated. Several highly significant findings have been identified that suggest an association between uncharacterized factors related to the soldier's perceived exposure potential, as recorded during a carefully administered questionnaire, and semen analysis. These findings are summarized in Table 64.

The inadequacies of the existing body of epidemiological data concerning male reproductive effects associated with lead exposures have been recently reviewed.[94] Alterations of male reproductive function consistent with those identified in Table 64 have been associated with workplace exposures to lead. Similar alterations of human male reproductive function have been reported in association with microwave exposure.[99]

In most reported cases of male reproductive effects, relatively high blood lead levels have been associated with adverse findings. The relationship between increasing lead exposures and adverse male reproductive effects reported by Lancranjan et.al.[12] has been reviewed.[94] Approximately 25 percent of the individuals categorized as "physiological lead absorption"

Table 64. Summary review of statistically significant findings related to semen analysis associated with historical exposure and subject statement of fertility concern. Entries are provided in the form of "p values" with values less than p = 0.05 considered significant. Occasional non-significant entries are provided for comparison with the alternative exposure group.

	POSSIBLE LEAD EXPOSURE			POSSIBLE MICROWAVE EXPOSURE		
		CONCERNED			CONCERNED	
VARIABLE	TOTAL	NO	YES	TOTAL	NO	YES
SPERM COUNT						
Manual Count	0.50			0.0085		
Adjusted			0.014		0.010	
Total Count	0.55			0.027		
Adjusted			0.067		0.051	
MORPHOLOGY						
Normal	0.92			0.20		
Adjusted			0.019			0.032
Absent head	0.20			0.98		
Adjusted		0.025				0.045
MOTILITY						
Percent	0.51			0.059		
Linearity	0.054			0.065		
Bt. Cr. Freq.	0.0077			0.044		
Adjusted		0.024	0.0017		0.012	0.013
VIABILITY						
Penetr. Index						
with outlier	0.040			0.12		
no outlier	0.32			0.0085		
ACCES. SEX GLND						
Semen Volume	0.95			0.52		
Fructose	0.027			0.91		

in that review demonstrated alterations involving spermatogenesis, asthenospermia, hypospermia, or teratospermia. These findings are consistent with those identified in the present study where statistically significant differences in sperm count and function were found at blood lead levels considered to be within physiological limits. The review may be used to support the biological feasibility of lead exposure related sequelae.

With reference to the findings of the present study, it is hypothetically possible that short-term, intermittent, high-dose lead exposures could result in demonstrable changes in male reproductive parameters. Since the male reproductive cycle requires approximately 90 days for completion, it is possible that a short-lived pharmacological insult during sperm development could be detected within that time. For example, toxic biochemical alterations in secondary sex gland functions would be detectible within days of exposure. In contrast, adverse sequelae of damage during the mitotic or meiotic germ cell divisions would not be observed in semen analysis until several weeks following the insult. As a consequence, it is hypothetically possible that blood lead levels and hematological indices could be within the clinically accepted normal range while the sperm analysis reveals a delayed response to the earlier insult. Short-lived, high-dose exposures to male reproductive spermatogenic toxicants could severely compromise a developing cohort of sperm cells and be observed as a transient change in sperm count, morphology, or function several months after the exposure.

CONCLUSIONS

Information identified in this study is preliminary in nature and must not be construed to represent a causal association between either actual lead or microwave exposure without additional study.

Use of mobile laboratory equipment afforded the opportunity to provide an on-site evaluation of male fecundity among artillerymen. Distinct advantages included the ability to perform almost immediate analyses of selected semen and sperm parameters and availability of the videotape recorder for subsequent computerized analysis.

Analysis of information provided by the soldiers with potential lead exposures studied in this population demonstrated participation in the study was correlated with concern for fathering difficulty. As a consequence of the possible bias of concern about parenting difficulty and variable abstinence times, statistical analyses incorporated those parameters as potential confounding variables. After adjustments, analysis of differences between the lead and microwave exposure groups were found to be statistically significant from the control group for potential reproductive effects in the two potentially "exposed" populations.

Although this study is a cross-sectional epidemiologic evaluation, the information gained provides valuable clinical information concerning these three groups of soldiers. Information gleaned from this study should afford the opportunity to improve the scientific merits and focus of future studies.

Historical exposure characterizations and weapons system specifications allowed only approximate exposure estimates which could not be confirmed based upon clinical laboratory analysis for blood lead level. Results of the laboratory and statistical analysis appear to be valid and reliable.

Adverse reproductive effects from chemical or physical exposure hazards are biologically feasible if exposures are excessive. The possibility of transient, readily reversible changes in biological function following high level, intermittent exposures appears possible.

Precautionary statements have been published concerning proper use of artillery and microwave systems. No further advisory statements appear to be indicated at this time.

Since the study findings appear scientifically reliable and are biologically feasible, the null hypothesis cannot be rejected. It is theoretically possible that exposure to lead or microwave associated with military duties could occur and result in adverse health effects.

RECOMMENDATIONS

Individuals with clinically important alterations in semen characteristics, e.g. sperm counts less than 20 million, should be afforded the opportunity to submit a repeat semen analysis and obtain medical evaluation.

Further study should be performed to validate the findings of the current study. Prudent scientific and medical practice would suggest that additional studies should be performed as soon as possible. Although the unexpected findings of semen parameters outside selected ranges should not be ignored, they should not be used to generate unnecessary concern among soldiers prior to further study.

The number of study subjects should be increased in future studies to assure improved statistical power.

Refinement of the standardized questionnaire to improve characterization of lead and microwave radiation potential exposures should be completed prior to initiation of additional studies.

Future questionnaires should be modified to better assess the exposure profiles of study subjects. For soldiers with a potential for lead exposure, questions should focus on type of weapons fired, frequency of firing in training, type of charge fired, actual duty assignment, availability and use of crew ballistic shelters, functional adequacy of the bore evacuator, and time duration since last firing was completed. Similarly, for soldiers potentially exposed to microwave, information concerning the actual microwave sources used, frequency of use, duration of use, and non-ionizing radiation characteristics of radiation spectrum and intensity should be obtained in the historical review.

Future studies concerning potential male reproductive effects should limit the number of biological parameters evaluated in the protocol. The number of parameters assessed in this study was substantial, which resulted in a significant delay in laboratory analysis, statistical analysis and data interpretation.

If possible, future studies should be performed in a time-triggered, prospective sequence based upon exposure profiles to identify possible cyclic changes among representative populations of soldiers. If possible, cyclic patterns of male reproductive function independent of lead and microwave exposures should be more optimally characterized using prospective studies.

If resources are adequate, and if logistically feasible, environmental measurements of exposure could be obtained simultaneously as a portion of the study protocol. If adverse health effects are confirmed or if airborne lead aerosol levels or microwave exposures significantly exceed excursion or acceptable TWA limits, a reasonable concern for hazardous exposure circumstances should be raised. Careful training and worker notification of potential hazard should be provided and documented to assure compliance with the hazard communication program.

Development of weapons systems of the future should attempt substitution for lead as a de-coppering agent, if possible.

Lead particulate filtration systems should be incorporated into weapons systems known to be associated with high lead aerosol exposures in confined spaces, e.g. crew cabins, if possible.

Appropriate personal protection should be used to limit exposures to excessive concentrations of lead in aerosols when substitution, elimination, or engineering control are not feasible. Surgical masks will not afford adequate protection, as a result of the aerosol particulate sizes.

Medical surveillance to evaluate individual soldiers for signs of adverse effects from exposure to physical agents or chemical substances should be carefully tailored. For exposures which have the potential to alter male reproductive function, semen analysis should be performed, if indicated. The availability of carefully performed and standardized semen analysis for concerned individuals with occupationally-related fecundity concerns should be recognized as a component of a complete medical evaluation.

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APPENDIX A

PROPOSAL
"LEAD EXPOSURE AND BIOLOGICAL RESPONSES IN MILITARY WEAPON SYSTEMS"

TECHNICAL PROPOSAL
FOR MODIFICATION OF
AN INTERAGENCY AGREEMENT

between the

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
and
U. S. DEPARTMENT OF ENERGY

Lead Exposure and Biological Responses in Military Weapon Systems

(U. S. Army Project Order No. 86PP6821)

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September 1988

Chronic Lead Exposures and Biological Responses in Career Artillerymen

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Specific Aims

(1) Determine, by means of *in vivo* x-ray fluorescence, the concentration of lead in the bones of artillerymen who have had 5, 10, or 15 years of artillery service, to provide a measure of cumulative lead exposure.

(2) Determine nerve conduction velocities and blood pressure in the same individuals, to evaluate possible responses to chronic lead exposures.

Background

Artillerymen are exposed by inhalation to aerosols containing lead when they fire lead-containing charges from armored vehicle weapons. In a recent study, scientists in our group at Argonne National Laboratory (ANL) determined that time-weighted average air lead concentrations ranged from 1-2 μg Pb/m^3 up to 600 μg Pb/m^3 at crew member positions of an 8-inch Howitzer during firing of zone 9 charges. The OSHA permissible exposure limit (PEL) for lead is 50 μg Pb/m^3 for an 8-h daily work period. Because the circumstances of military exposure to lead aerosols (sporadic, potentially high levels) differ from the circumstances of most civilian industrial exposures (continuous, predominantly low levels), military protection standards may need to be formulated differently from those recommended for industry. The need,

therefore, exists to evaluate military exposures to lead and to recommend exposure standards that will provide necessary protection both to career artillerymen and to crew members under combat conditions.

We are currently completing a study of acute lead exposures and responses among crew members who fired high-lead charges from 8-inch Howitzers at Fort Sill, OK. We now propose to study chronic lead exposures and responses among career artillerymen, who have potentially been exposed to weapons lead aerosols over much longer periods of time than the crewmembers in the acute exposure study.

Bone lead concentrations will be used as a measure of cumulative exposure to lead. Biological responses to be evaluated are changes in nerve conduction velocities and blood pressure. Decreases in nerve conduction velocity have been observed among persons occupationally exposed to lead (1-7). This response was chosen for study because the nervous system is critical to the effective performance of the duties of an artilleryman. Lead exposure has also been associated with a rise in blood pressure (8). This response will be investigated because recent studies have focused on a blood pressure response to low levels of lead exposure (9), blood pressure can readily be measured, and increases in blood pressure could influence the long-term health of persons with careers in the artillery. Of interest may be the observation from our Acute Lead Exposure Study at Fort Sill that one of the very few members of that study with an extended term in the artillery also had consistently lower nerve conduction velocities than most of the other persons in the study.

Experiment Design and Methods

Overall design. The following questions will be addressed:

(1) Do career artillerymen have bone lead concentrations that are higher than those of a control group not in the artillery? Do their bone lead concentrations increase with increasing length of service in the artillery?

(2) Are nerve conduction velocities lower or blood pressure values higher in the career artillerymen than in the control groups?

(3) Do nerve conduction velocities decrease either with increased bone lead concentration or with increased length of service in the artillery?

(4) Does blood pressure increase either with increased bone lead concentration or with increased length of service in the artillery?

Three groups of artillerymen (~20 men/group) will be selected at random from groups identified with averages of 5, 10, or 15 years of artillery service. Three corresponding groups of appropriately chosen control service men will also be identified. The control groups will be identified with input from contracting officer, Major David Parmer. Ideally, they will be made up of service men who are similar to the artillerymen in age, race, socioeconomic status, and length of military service but who have had substantially less exposure to weapons firing. The study as currently planned will thus include approximately 60 artillerymen and 60 control service men.

Nerve conduction velocity measurements. The velocities of six nerves (three sensory and three motor nerves) will be determined in each study subject according to the procedures used in our Acute Lead Exposure Study at Fort Sill. The nerve measurements will take approximately one hour per study subject, and a maximum of six subjects will be measured per day. The nerve measurements will therefore take approximately 20 measurement days to complete.

Bone lead concentration measurements. Bone lead concentrations will be determined in the tibia (cortical bone) and possibly also the calcaneus (trabecular bone of the heel) by an *in vivo* x-ray fluorescence technique discussed in detail by Somervaille et al. (10-13). This method uses the gamma rays from a cadmium-109 source to induce the emission of characteristic lead K x-rays from the lead deposited in bone.

The apparatus consists of a radiation source, a radiation detector, associated electronics, calibration standards and positioning devices for the subject's body. The radiation detector is a high resolution, transportable, germanium crystal for x-ray detection attached to a dewar containing liquid nitrogen. The germanium crystal is housed in a cylindrical package. The cadmium-109 source is in the form of a ring and is mounted concentric to the housing. There is shielding between the radiation source and the detector to prevent the gamma rays from the source from entering the detector. Electrical pulses from the detector corresponding to the energies of the lead x-rays are amplified and fed into a multichannel analyzer for energy sorting and storage. Spectrum resolution and data analyses are carried out on a computer.

The method described above was chosen after careful comparison of the several methods currently being used for making *in vivo* bone lead measurements (14-17). The lower limit of detection for the instrument being used by Somervaille et al. is 3-4 μg Pb/g wet bone (L. Somervaille, personal communication). Lead concentrations in the bones of environmentally-exposed persons range from 5 to 30 μg Pb/g. Excellent agreement has been obtained between lead concentrations in human tibia samples determined by cadmium-109 x-ray fluorescence *vs.* atomic absorption spectrophotometry (11), demonstrating the validity of the fluorescence method for measuring total bone lead concentrations.

We plan to fabricate an instrument according to the specifications of the Somervaille instrument. Calibration standards will be exchanged with Dr. Somervaille to provide a direct comparison of our respective measurement systems. Fabrication will be carried out by staff members at Argonne who collectively have in-depth knowledge in all of the areas associated with x-ray fluorescence spectroscopy, e.g., electronics of the instrument, optimal shielding of the source and detector, computerized data processing required to achieve low limits of detection, and measurement of radiation dose to the subject. We estimate that it will take 8-10 months to fabricate the instrument and become proficient in its use.

Each in vivo bone lead determination will take approximately 40 minutes. During this time, the subject will fill out a questionnaire on the instrument's computer. The maximum radiation dose to a subject's leg will be approximately 1000 times lower than the annual limit for occupational radiation exposure to the leg set by the International Commission on Radiological Protection. About 20 days will be required to make measurements on 120 subjects.

Blood samples will also be taken from each subject for determination of blood lead concentration. These data will allow comparison of our study population with other populations in which both blood lead and bone lead concentrations were determined.

Blood pressure measurements. Lead exposure has been associated with hypertension for many years (8,18-21), although the subject remains controversial (22-23). Because our study population includes persons potentially exposed to lead over a number of years, and because blood pressure is relatively easy to assess, we plan to determine the blood pressure of the persons in our study and to determine by questionnaire the incidence of diagnosed hypertension.

Human subjects

Attached is the consent form prepared for this study. This form has been approved by the ANL Human Use Review Committee. As indicated, all information obtained from this study that carries personal identifiers will be kept in locked files, and all reports will present results in such a form that they do not reveal any study subject's personal identity. Participation in the study will be voluntary.

Schedule for Completion of Tasks

Figure 1 shows a Gantt chart describing the individual tasks associated with this study and the anticipated schedule for the completion of each task. It is anticipated that the entire study will take approximately 1.5 years to complete.

Budget

See attached.

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APPENDIX B

PARTICIPANT QUESIONNAIRE

ID #: (1-4)

TODAY'S DATE: _____ _____ _____ (5-10)
(Year) (Month) (Day)

(Interviewer: Read sections in capital letters only).

YOUR ANSWERS TO THESE QUESTIONS WILL BE CODED WITH YOUR ID NUMBER AND NOT YOUR NAME. ALL OF THE ANSWERS YOU GIVE TO ME WILL BE KEPT CONFIDENTIAL AND USED FOR RESEARCH ONLY. IF ANY OF THE QUESTIONS I ASK YOU ARE NOT CLEAR, PLEASE STOP ME, AND I WILL TRY TO MAKE THEM CLEAR.

1. WHAT IS YOUR DATE OF BIRTH? _____ _____ _____ (11-16)
(Year) (Month) (Day)

2. WHAT IS YOUR RACE/ETHNIC BACKGROUND? (17)

White (not of Hispanic origin) ____1
Black (not of Hispanic origin) ____2
Hispanic ____3
American Indian or Alaskan Native ____4
Asian or Pacific Islander:
Indian, Pakistani ____5
Pacific Islander ____6
Other Asian ____7

3. WHAT IS YOUR JOB TITLE? _____

4. WHAT DO YOU DO IN YOUR CURRENT JOB? _____

_____

_____

_____

_____ (18-19)

ID #:

5. DO YOU USE OR REPAIR ARTILLERY? (20)

No (Skip to #6)	____0
Yes, use (Go to #5a-b)	____1
Yes, repair (Go to #5a-b)	____2
Yes, use and repair (Go to#5a-b)	____3

If "yes" to #5, answer #5a and b below:

a. HOW MUCH TIME DURING THE WEEK DO YOU SPEND WORKING WITH ARTILLERY? (21)

35 or more hours a week (full time)	____1
5-34 hours a week (part time)	____2
(Specify_____)	
Less than 5 hours or occasionally	____3

b. IS THIS THE ONLY KIND OF WORK YOU HAVE DONE IN THE LAST 3 MONTHS? (22)

No (Go to b(1)	____0
Yes (Skip to #6)	____1

If "no" to #5b, answer (1) below:

(1) WHAT OTHER KINDS OF WORK HAVE YOU DONE IN THE LAST 3 MONTHS? _____ (23)

_____

_____

6. IN WHAT MONTH AND YEAR DID YOU START YOUR CURRENT JOB IF UNSURE OF DATE, PLEASE MAKE YOUR BEST GUESS. _____ _____ (24-27)
(Year) (Month)

7. DID YOU TAKE A VACATION, LEAVE OF ABSENCE OR SICK LEAVE LONGER THAN 4 WORKING DAYS IN THE LAST 3 MONTHS? (28)

No (Skip to #8)	____0
Yes (Go to #7a-b)	____1

If "yes" to #7, answer #7a-b below.

a. SPECIFY _____ ____ (29)

b. HOW MANY DAYS? _____ (30-32)

8. ARE YOU EXPOSED TO ANY CHEMICALS (INCLUDING LEAD AND SOLDER), MICROWAVES (INCLUDING RADAR), HEAT, OR RADIATION IN YOUR CURRENT JOB? (33)

No (Skip to #9)	____0
Yes (Specify below	____1

_____ ___ (34)

ID #:

9. DESCRIBE YOUR LAST JOB, INCLUDING MILITARY EXPERIENCE. ___ (35)

_____

_____

10. WHEN DID YOU START THIS JOB? _____ (Year) _____ (Month) (36-39)

11. WHEN DID THIS JOB END? _____ (Year) _____ (Month) (40-43)

12. WERE YOU EXPOSED TO ANY CHEMICALS (INCLUDING LEAD AND SOLDER), MICROWAVES (INCLUDING RADAR), HEAT, OR RADIATION IN THAT JOB? (44)

No (Skip to #13) ____0

Yes (Specify below) ____1

_____

_____ ___ (45)

13. DESCRIBE YOUR NEXT-TO-LAST JOB. ___ (46)

_____

_____

14. WHEN DID YOU START THIS JOB? _____ (Year) _____ (Month) (47-50)

15. WHEN DID THIS JOB END? _____ (Year) _____ (Month) (51-54)

16. WERE YOU EXPOSED TO ANY CHEMICALS (INCLUDING LEAD AND SOLDER) MICROWAVES (INCLUDING RADAR), HEAT OR RADIATION IN THAT JOB? (55)

No (Skip to #17) ____0

Yes (Specify below) ____1

_____

_____ ___ (56)

ID #:

17. DESCRIBE YOUR JOB BEFORE THAT. ___ (57)

_____

_____

18. WHEN DID YOU START THIS JOB? _____ (Year) _____ (Month) (58-61)

19. WHEN DID THIS JOB END? _____ (Year) _____ (Month) (62-65)

20. WERE YOU EXPOSED TO ANY CHEMICALS (INCLUDING LEAD AND SOLDER), MICROWAVES (INCLUDING RADAR), HEAT, OR RADIATION IN THAT JOB? (66)

No (Skip to #21) ____0
Yes (Specify below) ____1

_____

_____ ___ (67)

Card 0 1 (70-72)

ID #:

21. DO YOU HAVE ANY HOBBIES, SECOND JOBS, OR ACTIVITIES WHICH MAY EXPOSE YOU TO: HEAT, CHEMICALS (INCLUDING LEAD AND SOLDER), MICROWAVES (INCLUDING RADAR), GLUES, EPOXY RESINS, PAINTS, SOLVENTS, CUTTING FLUIDS, OIL, OR PESTICIDES. (5)

No (Skip to #22) ____0
Yes (Go to #21a-c) ____1

If "yes" to #21, list hobbies below:

a. Hobby _____ ____ (6)
Year Started _____ Year Ended _____
Exposures _____
Frequency (times/year)_____

b. Hobby _____ ____ (7)
Year Started _____ Year Ended _____
Exposures _____
Frequency (times/year)_____

c. Hobby _____ ____ (8)
Year Started _____ Year Ended _____
Exposures _____
Frequency (times/year)_____

22. DID YOU SERVE IN THE MILITARY IN VIETNAM OR SOUTHEAST ASIA? (9)

No (Skip to #23) ____0
Yes (Go to #22a-c) ____1

If "yes to #22, answer #22a-c below.

a. WHAT MONTH AND YEAR DID YOUR SERVICE START? _____ _____ (10-13)
(Year) (Month)

b. WHAT MONTH AND YEAR DID YOUR SERVICE END? _____ _____ (14-17)
(Year) (Month)

c. DID YOU SPRAY OR WERE YOU EVER IN THE AREA SPRAYED WITH DEFOLIANTS? (18)

No ____0
Yes ____1
(Specify _____)
DK/NA ____9

23. DO YOU SMOKE, CHEW, OR USE SNUFF NOW; DID YOU QUIT; OR ARE YOU A NONSMOKER/NONUSER? (19)

I still smoke/chew/use snuff (Skip to #25) ____1
I quit (Go to #24) ____2
Nonsmoker/nonuser (Skip to #26) ____3

ID #:

24. WHEN DID YOU QUIT SMOKING? _____ (Year) _____ (Month) (20-23)

25. IN THE PAST YEAR (OR IN THE LAST YEAR) THAT YOU USED TOBACCO, HOW MUCH DID YOU AVERAGE PER DAY?
(Interviewer: If less than 1 per day, enter "-1")

a. # CIGARETTES PER DAY? _____ (24-25)

b. # CIGARS PER DAY? _____ (26-27)

c. # PIPEFULS OF TOBACCO PER DAY? _____ (28-29)

d. # UNITS SNUFF/CHEWING TOBACCO PER DAY?
(Specify units_____) _____ (30-31)

26. (Interviewer: Read slowly.)
THINK OF 3 GOOD FRIENDS. DON'T MENTION THEIR NAMES, JUST GET THEM IN MIND.

(Interviewer: Pause.)
DO YOU HAVE 3 FRIENDS IN MIND? AS FAR AS YOU KNOW, HOW MANY OF THEM SMOKE MARIJUANA OR HASHISH REGULARLY?

(Interviewer: Enter 0-3 or 9 for missing.) ___ (32)

27. WHAT BEST DESCRIBES YOUR DRINKING BEHAVIOR OVER THE LAST YEAR? YOU CAN GIVE MORE THAN ONE ANSWER. _____ (33-34)

"I . . . "

OCCASIONALLY GO ON A BINGE	____1
DRINK ALMOST EVERY DAY	____2
DRINK A FEW TIMES A WEEK	____3
DRINK OCCASIONALLY	____4
DRINK ALMOST NEVER	____5
NEVER DRINK (Skip to #29)	____6
NO ANSWER/NOT SURE (Skip to #29)	____9

28. THINK OF THE TIMES YOU HAD WINE, BEER, AND LIQUOR IN THE PAST YEAR. WHEN YOU DRINK, HOW MUCH DO YOU USUALLY HAVE AT ONE TIME?
(Interviewer: If less than 1, enter "-1")

a. HOW MANY BOTTLES OF BEER? _____ (35-36)

b. HOW MANY GLASSES OF WINE? _____ (37-38)

c. HOW MANY SHOTS (OUNCES) OF LIQUOR? _____ (39-40)

ID #:

29. IN THE LAST 3 MONTHS, HOW MANY CUPS OF CAFFEINATED COFFEE DID YOU DRINK ON AN AVERAGE DAY?
(Interviewer: If less than 1, enter "-1") _____ (41-42)

30. IN THE LAST 3 MONTHS, HOW MANY CUPS OF CAFFEINATED TEA DID YOU DRINK ON AN AVERAGE DAY?
(Interviewer: If less than 1, enter "-1") _____ (43-44)

31. IN THE LAST 3 MONTHS, HOW MANY BOTTLES OR CANS OF CAFFEINATED COLA OR SODA DID YOU DRINK ON AN AVERAGE DAY?
(Interviewer: If less than 1, enter "-1") _____ (45-46)

32. IN THE LAST 3 MONTHS, HOW FREQUENTLY DID YOU TAKE VERY HOT BATHS, SAUNAS, OR STEAM BATHS (DO NOT INCLUDE SHOWERS) ON THE AVERAGE? (47)

Never	____0
1-3 times a month	____1
1-3 times a week	____2
4 or more times a week	____3
DK/NA	____9

33. IN THE LAST 3 MONTHS, WHAT TYPE OF UNDERWEAR DID YOU USUALLY WEAR? (48)

Boxer shorts	____1
Briefs, jockey or bikinis	____2
No underwear	____3
DK/NA	____9

34. IN THE LAST 3 MONTHS, HOW FREQUENTLY DID YOU SLEEP IN YOUR UNDERWEAR? (49)

Never	____0
Less than once a month	____1
1-3 times a month	____2
1-3 times a week	____3
4 or more times a week	____4
DK/NA	____9

35. IN THE LAST 3 MONTHS, HAVE YOU HAD ANY VIRAL OR BACTERIAL INFECTIONS OR FLU THAT CAUSED A FEVER? (50)

No (Skip to #36)	____0
Yes (Go to #35a-b)	____1
DK/NA (Skip to #36)	____9

<u>If "yes" to #35, answer #35a-b below:</u>

a. PLEASE DESCRIBE THE INFECTION. _____

b. WHAT MONTH DID YOU HAVE THIS INFECTION? _____ (51-52)

ID #:

36. IN THE LAST 3 MONTHS, DID YOU HAVE ANY X-RAYS OTHER THAN DENTAL X-RAYS? (53)

No (Skip to #37) ____0
Yes (Go to #36a-b) ____1
DK/NA (Skip to #37) ____9

If "yes" to #36, answer #36a-b below:

a. PLEASE SPECIFY THE TYPE OF X-RAY. _____

b. WHEN DID YOU HAVE YOUR MOST RECENT X-RAY? _____ (Year) _____ (Month) (54-57)

37. WERE YOU EVER DIAGNOSED AS HAVING ANY OF THESE MEDICAL CONDITIONS? IF UNSURE OF DATE, PLEASE MAKE YOUR BEST GUESS.

	NO	YES	DK/NA		YEAR DIAGNOSED	DATE OF LAST TREATMENT	TREATMENT
a. MUMPS	0	1	9	(58)	_____		
b. SICKLE CELL ANEMIA	0	1	9	(59)	_____		
c. CYSTIC FIBROSIS	0	1	9	(60)	_____		
d. CHROMOSOMAL (GENE) PROBLEM	0	1	9	(61)	_____		
e. ALCOHOLISM	0	1	9	(62)	_____		
f. HIGH BLOOD PRESSURE	0	1	9	(63)	_____	_____	_____
g. EPILEPSY	0	1	9	(64)	_____	_____	_____
h. BOWEL DISEASE (Specify_____)	0	1	9	(65)	_____	_____	_____
i. DIABETES	0	1	9	(66)	_____	_____	_____

CARD 0 2 (70-72)

ID #:

38. HAVE YOU EVER BEEN TREATED FOR CANCER? (5)

No (Skip to #39) ____0
Yes (Go to #38a-b) ____1
DK/NA (Skip to #39) ____9

If "yes" to #38, answer #38a-b below:

a. WHAT TYPE OF CANCER? _____ _____ (6-7)

b. WHAT KIND OF TREATMENT AND WHEN?

				Date of Treatments
(1) DRUGS/CHEMOTHERAPY	0____No	1____Yes	(8)	_____
(2) RADIATION THERAPY	0____No	1____Yes	(9)	_____
(3) SURGERY	0____No	1____Yes	(10)	_____

39. WERE YOU BORN WITH ANY BIRTH DEFECTS? (11)

No (Skip to #40) ____0
Yes (Go to 39a-b) ____1
DK/NA (Skip to #40) ____9

If "yes" to #39, answer #39a-b:

a. WHAT WAS THE CONDITION? _____

b. WHAT WAS THE TREATMENT? _____

40. HAVE YOU EVER BEEN DIAGNOSED AS HAVING ANY OTHER MAJOR ILLNESS OR CONDITION? (12)

No (Skip to #41) ____0
Yes (Go to 40a-b) ____1
DK/NA (Skip to #41) ____9

If "yes" to #40, answer #40a-b:

a. WHAT WAS THE CONDITION? _____

b. WHAT WAS THE TREATMENT? _____

ID #:

41. HAVE YOU TAKEN ANY OTHER PRESCRIPTION OR NON-PRESCRIPTION MEDICATIONS IN THE PAST YEAR? (13)

No (Skip to #42)	____0
Yes (Go to 41a-c)	____1
DK/NA (Skip to #42)	____9

If "yes" to #41, list medications below:

	WHAT MEDICATIONS?	FOR WHAT?	WHEN WAS IT STARTED?	DO YOU TAKE IT NOW?	
a.	_____	_____	_____	No____0 Yes____1	(14-15)
b.	_____	_____	_____	No____0 Yes____1	(16-17)
c.	_____	_____	_____	No____0 Yes____1	(18-19)

42. DURING THE LAST 6 MONTHS, ABOUT HOW MANY TIMES A WEEK DID YOU EJACULATE (THROUGH INTERCOURSE OR MASTURBATION)? _____ (20-21)
(Interviewer: Code "99" for DK/NA & "-1" if less than once/week)

43. IN THIS STUDY, IT WILL BE NECESSARY TO COLLECT A SEMEN SAMPLE BY MASTURBATION. HOW COMFORTABLE ARE YOU WITH THIS METHOD OF OBTAINING SEMEN? ARE YOU . . . (22)

VERY COMFORTABLE	____1
SOMEWHAT COMFORTABLE	____2
SOMEWHAT UNCOMFORTABLE, OR	____3
VERY UNCOMFORTABLE BUT WILLING TO TRY	____4
(Don't Know/No Answer)	____9

44. HAVE YOU EVER THOUGHT YOU HAD TROUBLE FATHERING A CHILD (A FERTILITY PROBLEM)? (23)

No (Skip to #45)	____0
Yes (Go to #44a-b)	____1
DK/NA (Skip to #45)	____9

If "yes" to #44, answer 44a-b:

a. PLEASE SPECIFY: _____

b. WHEN? _____

ID #:

45. HAVE YOU EVER CONSULTED A DOCTOR OR CLINIC BECAUSE YOU THOUGHT YOU MIGHT HAVE A FERTILITY PROBLEM? (24)

No (Skip to #46)	____0
Yes, myself (Go to #45a-c)	____1
Yes, my partner/wife only (Go to #45a-c)	____2

If "yes" to #45, answer #45a-c below:

a. WHAT WAS THE MONTH AND YEAR YOU FIRST CONSULTED A DOCTOR ABOUT A POSSIBLE FERTILITY PROBLEM?
IF UNSURE OF DATE, PLEASE MAKE YOUR BEST GUESS. (25-28)

_____ (Year) _____ (Month)

b. THE VISIT WAS MADE BY: (29)

Myself	____1
My partner	____2
Myself and my partner	____3

c. WERE YOU EVER DIAGNOSED AS HAVING A FERTILITY PROBLEM? (30)

No (Skip to #46)	____0
Yes, myself (Go to #c(1)(2)	____1
Yes, my partner/wife only (Go to #c(1)(2)	____2

If "yes" to #45c, answer (1) & (2) below:

(1) WHAT DID THE DOCTOR SAY? _____

(2) DID YOU (SHE) GET ANY TREATMENT FOR YOUR FERTILITY PROBLEM AT THAT OR AFTER? _____ (31)

No (Skip to #46)	____0
Yes (Go to (a) & (b))	____1

If "yes" to #(2), answer (a) & (b) below:

(a) WHAT KIND OF TREATMENT? _____

(b) WHEN (Month & Year)? _____

46. HAVE YOU EVER HAD A SPERM ANALYSIS? (32)

No (Skip to #47)	____0
Yes (Go to #46a-c)	____1
DK/NA (Skip to #47)	____2

If "yes" to #46, answer #46a-c below:

a. WAS YOUR SPERM ANALYSIS FOR A VASECTOMY? (33)

No	____0
Yes	____1
DK/NA	____9

ID #:

b. WHAT MONTH AND YEAR DID YOU HAVE YOUR FIRST SPERM ANALYSIS?
IF UNSURE OF DATE, PLEASE MAKE YOUR BEST GUESS.

(34-37)

_____ (Year) _____ (Month)

c. WHAT WERE THE RESULTS OF YOUR FIRST SPERM ANALYSIS? (38)

Normal	____0
Abnormal but don't know the specifics	____1
Low sperm count	____2
Poor motility	____3
High count	____4
Low volume	____5
Abnormal shapes	____6
Poor speed (velocity)	____7
Other (Specify_____)	____8
DK/NA	____9

47. WERE YOU EVER DIAGNOSED AS HAVING ANY OF THE FOLLOWING CONDITIONS OR SURGERIES?

	NO	YES	DK/NA		YEAR DIAGNOSED	YEAR OF SURGERY
a. ONLY ONE TESTICLE (Undescended testicles)?	0	1	9	(39)	_____	_____
b. A LUMP IN THE SCROTUM (Hydrocele)?	0	1	9	(40)	_____	_____
c. VARICOSE VEINS OF THE SCROTUM (Varicocele)?	0	1	9	(41)	_____	_____
d. CIRCUMCISION?	0	1	9	(42)	_____	_____
e. A FORESKIN THAT CAN'T BE PULLED BACK (Phimosis)?	0	1	9	(43)	_____	_____
f. TESTICLE BIOPSY?	0	1	9	(44)	_____	_____
g. VASECTOMY?	0	1	9	(45)	_____	_____
h. VASECTOMY REPAIR?	0	1	9	(46)	_____	_____
i. REMOVAL OF APPENDIX?	0	1	9	(47)	_____	_____
j. HERNIA REPAIR?	0	1	9	(48)	_____	_____

ID #:

48. WERE YOU EVER DIAGNOSED AS HAVING ANY OF THESE CONDITIONS?

	NO	YES	DK/NA		YEAR DIAGNOSED	YEAR OF SURGERY
a. URINARY TRACT INFECTION? (Specify site_____)	0	1	9	(49)	_____	_____
b. NON-SPECIFIC URETHRITIS OR DISCHARGE FROM PENIS?	0	1	9	(50)	_____	_____
c. INFECTION OF THE TESTICLE (Orchitis)?	0	1	9	(51)	_____	_____
d. INFECTION OF THE PROSTATE GLAND (Prostatitis)?	0	1	9	(52)	_____	_____
e. INFECTION OF THE SEMINAL VESICLES (Vesiculitis)?	0	1	9	(53)	_____	_____
f. INFECTION OF THE EPIDIDYMIS (Epididymitis)?	0	1	9	(54)	_____	_____
g. CHLAMYDIA?	0	1	9	(55)	_____	_____
h. SYPHILIS (Lues)?	0	1	9	(56)	_____	_____
i. GONORRHEA (Clap, Dose)?	0	1	9	(57)	_____	_____
j. GENITAL HERPES?	0	1	9	(58)	_____	_____

49. HAVE YOU HAD ANY OTHER UROLOGICAL CONDITION?

No ____0 (59)
Yes (Specify below) ____1

_____

50. HAVE YOU HAD ANY GENITAL INJURIES FOR WHICH YOU NEEDED MEDICAL HELP?

No (Skip to #51) ____0 (60)
Yes (Go to #50a-b) ____1

If "yes" to #50, answer #50a-b:

a. PLEASE DESCRIBE YOUR INJURY: _____

b. WHEN DID IT HAPPEN (Year)? _____

ID #:

51. HOW MANY PREGNANCIES HAVE YOU FATHERED? PLEASE INCLUDE PREGNANCIES THAT RESULTED IN LIVE BIRTH, STILLBIRTH, MISCARRIAGE, INDUCED ABORTION AND TUBAL (ECTOPIC) PREGNANCY?
(If none, skip to #53) _____ (61-62)

CARD 0 3 (70-72)

52. I WOULD LIKE TO KNOW ABOUT EACH OF THESE PREGNANCIES. STARTING WITH YOUR LAST PREGNANCY, TELL WHAT HAPPENED WITH EACH PREGNANCY AND THE DATE OF THE OUTCOME.
(Interviewer: In the case of multiple births (twins, triplets, etc.) list each birth separately.)

Partner Number Codes

1. Most recent partner
2. Prior partner
3. Partner prior to #2

Pregnancy Outcome Codes

1. Live birth
2. Stillbirth
3. Induced abortion
4. Miscarriage
5. Tubal pregnancy

PREGNANCY NUMBER		PARTNER NUMBER		PREGNANCY OUTCOME	ANY MEDICAL PROBLEM IN NEWBORN?		BIRTH YEAR	
___	(5-6)	___ (7)	___	_____	___	(9)	_____	(10-11)
___	(12-13)	___ (14)	___	_____	___	(16)	_____	(17-18)
___	(19-20)	___ (21)	___	_____	___	(23)	_____	(24-25)
___	(26-27)	___ (28)	___	_____	___	(30)	_____	(31-32)
___	(33-34)	___ (35)	___	_____	___	(37)	_____	(38-39)
___	(40-41)	___ (42)	___	_____	___	(44)	_____	(45-46)
___	(47-48)	___ (49)	___	_____	___	(51)	_____	(52-53)
___	(54-55)	___ (56)	___	_____	___	(58)	_____	(59-60)
___	(61-62)	___ (63)	___	_____	___	(65)	_____	(66-67)

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ID #:

53. DID YOUR MOTHER TAKE DES (DIETHYSTILBESTROL) OR OTHER HORMONE TO PREVENT MISCARRIAGE DURING THE TIME THAT SHE WAS PREGNANT WITH YOU?

No	____0	(5)
Yes, DES	____1	
Yes, other hormone	____2	
DK/NA	____9	

54. CAN YOU THINK OF ANYTHING ELSE WHICH MAY BE IMPORTANT FOR US TO KNOW?

No	____0	(6)
Yes (Specify below)	____1	

_____

55. WHAT IS YOUR ONE MOST IMPORTANT REASON FOR VOLUNTEERING FOR OUR STUDY?

_____

_____ _____ (7-8)

56. MEN HAVE SIGNED UP FOR OUR STUDY FOR MANY REASONS. PLEASE RATE EACH REASON I READ TO YOU AS "NOT IMPORTANT," SOMEWHAT IMPORTANT," OR "VERY IMPORTANT" TO YOU AS A REASON THAT YOU SIGNED UP.

	NOT IMPORTANT	SOMEWHAT IMPORTANT	VERY IMPORTANT	
a. ADD TO SCIENTIFIC KNOWLEDGE	0	1	2	(9)
b. I CAN USE THE MONEY	0	1	2	(10)
c. MANY OF MY COWORKERS/WORK FRIENDS ARE IN THE STUDY	0	1	2	(11)
d. IT MAY HELP ME TO KNOW MY TEST RESULTS	0	1	2	(12)
e. I FEEL THAT IT MAY HELP ME TO LEARN WHY I AM HAVING TROUBLE FATHERING CHILDREN	0	1	2	(13)
f. ANYTHING ELSE (Specify below)	0	1	2	(14)

_____

ADDITIONAL COMMENTS:

_____ (15-40)

_____ (41-69)

CARD 0 5 (70-72)

ID#:

57. DO YOU USE OR CLEAN FIREARMS OR WEAPONS OTHER THAN ARTILLERY? (SPECIFY USE AND/OR CLEAN.)

Never	____
Less than 2 hours per week	____
2-20 hours per week	____
More than 20 hours per week	____

58. DO YOU USE OR VISIT OUTDOOR FIRING RANGES?

Never	____
Less than 2 hours per week	____
2-20 hours per week	____
More than 20 hours per week	____

59. DO YOU USE OR VISIT INDOOR FIRING RANGES?

Never	____
Less than 2 hours per week	____
2-20 hours per week	____
More than 20 hours per week	____

APPENDIX C

NONPARTICIPANT QUESTIONNAIRE

NONPARTICIPANT CHARACTERIZATION

SEQUENCE #: _____ (1-3)

INTERVIEWER: _____

EVEN THOUGH YOU DO NOT WANT TO BE IN OUR STUDY, WE WOULD LIKE TO ASK YOU SIX SHORT QUESTIONS BECAUSE WE NEED TO KNOW WHETHER THE MEN WHO ARE IN THE STUDY ARE SIMILAR TO OR DIFFERENT FROM THOSE WHO DON'T WANT TO BE IN IT IN VERY GENERAL WAYS.

1. WHAT IS YOUR DATE OF BIRTH? _____ _____ _____ (4-9)

(Year) (Month) (Day)

2. WHAT IS YOUR RACE/ETHNIC BACKGROUND? (10)

White (not of Hispanic origin) ____1
Black (not of Hispanic origin) ____2
Hispanic ____3
American Indian or Alaskan Native ____4
Asian or Pacific Islander:
Indian, Pakistani ____5
Pacific Islander ____6
Other Asian ____7

3. HOW MANY PREGNANCIES (NORMAL AND MISCARRIAGES HAVE YOU FATHERED? _____ (11-12)

a. HOW MANY OF THESE WERE NORMAL BIRTHS? _____ (13-14)

4. HAVE YOU EVER HAD TROUBLE FATHERING A CHILD (A FERTILITY PROBLEM)? (15)

No ____1
Yes (Specify below) ____2
DK/NA ____3

SPECIFY: _____

5. WHAT IS YOUR MOST IMPORTANT REASON FOR NOT PARTICIPATING IN OUR STUDY? (Specify below)

_____

_____ (16-17)

SEQUENCE #: _____

6. MEN DON'T WANT TO BE IN OUR STUDY FOR MANY REASONS. PLEASE RATE EACH OF THE REASONS I READ TO YOU AS "NOT IMPORTANT," "SOMEWHAT IMPORTANT," OR "VERY IMPORTANT" TO YOU AS A REASON THAT YOU DID NOT SIGN UP.

	NI	SI	VI	
a. I DON'T BELIEVE THIS STUDY IS WORTHWHILE.	1	2	3	(18)
b. I DON'T NEED THE MONEY.	1	2	3	(19)
c. I DON'T WANT TO HAVE A PHYSICAL OR TO GIVE A BLOOD SAMPLE.	1	2	3	(20)
d. I DON'T WANT TO MASTURBATE.	1	2	3	(21)
e. I THINK I MAY HAVE PROBLEMS FATHERING CHILDREN.	1	2	3	(22)
f. I DON'T WANT TO KNOW MY TEST RESULTS.	1	2	3	(23)
g. I DON'T LIKE INTERVIEWS.	1	2	3	(24)

ADDITIONAL COMMENTS:

_____ (25-46)

_____ (47-69)

CARD _____ (70-72)

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